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THE
MICROSCOPE

By

SIMON HENRY GAGE

Late Professor of Histology and Embryology
in Cornell University

SEVENTEENTH
EDITION: REVISED

ITHACA · NEW YORK
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To
the memory of
THEOBALD SMITH
pupil, friend, and
master investigator who
opened new paths to the
human mind.

PREFACE

In revising the matter for this, the seventeenth, edition of *The Microscope* changes have been made in every chapter in text and often in illustrations to render the subject more easily understood.

Attention has been called to the newly devised Electron Microscope with its greatly increased magnifying power and resolution over the ordinary microscope; to Polaroid for the micro-polariscope; to some new plastics for mounting in place of Canada balsam; to the high-pressure mercury lamps for ultra-violet radiation and the bright mercury lines for photographing objects with the microscope.

In general, however, the book retains its former character, and it is hoped that it will continue to serve students and users of the microscope in understanding the underlying principles involved in microscopical work.

As a final word, I wish to express my thanks and appreciation for the new cuts loaned and other aid rendered by the American manufacturers, the Bausch & Lomb Optical Company of Rochester and the Spencer Lens Company of Buffalo. The heads of those companies, Edward Bausch and Harvey N. Ott, have been my friends and advisers for many years and have been ever ready to help me over the rough places in my microscopical career.

Likewise it is a pleasure to render thanks to my University colleagues and to my fellow microscopists for their friendly interest and help; also to Clara Starrett Gage, Ph.D. for aid in preparing illustrations, making corrections and revising the index for this edition.

SIMON HENRY GAGE

May 20, 1941

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THE MICROSCOPE AND MICROSCOPICAL METHODS

INTRODUCTION

In dealing with the possibilities and use of any method of investigation, any machine or piece of scientific apparatus, the writer or teacher will naturally proceed as seems to him best from his personal experience, from his general theory of education, and from his conception of the style and method of presentation which will render his book most helpful and acceptable to his possible readers.

As stated in the preface to the sixth edition, this book had its origin in the laboratory, and its purpose was, and still is, to give the guidance by which those unfamiliar with the microscope and the methods of work with it can gain an intelligent understanding of the instrument, its limitations, and its possibilities for aiding one to arrive at truth. It has also the added purpose of bringing together the scattered information concerning new apparatus and method so that older workers may make use of them with a minimum amount of time and effort.

In working out the plan the following landmarks have been kept constantly in sight:

(1) To most minds, and certainly to those having any grade of originality, there is a great satisfaction in understanding principles; and it is only when the principles are firmly grasped that there is complete mastery of instruments, and full certainty and facility in using them. The same is true of the methods of preparing objects for microscopic study, and the interpretation of their appearances when seen under the microscope.

Much good work can be and has been done by the rule of thumb method, in which there is no real understanding of the underlying reason for any of the operations; the worker simply knows that good results follow a certain course of action. Probably most of the work of the world is done by rule of thumb. But for the highest creative work from which arises real progress both in theory and in practice, a knowledge of principles is indispensable.

(2) Need of abundant practical work to go with the theoretical part has been shown by all human experience. In all the crafts and in all the fine arts mastery comes only with almost endless effort and repetition, the most common example being the attainment of facility in music. Hence in this work there have been introduced many practical exercises so that the worker might gain the deftness needed. It is also a part of human experience that in successfully going through the manipulations necessary to demonstrate principles, there is acquired not only skill in experiment, but an added grasp of the principles involved.

After observing the work of students in my own and in other laboratories, the conclusion was reached, and expressed in the third edition of this book (1891) that "simply reading a work on the microscope, and looking a few times into an instrument completely adjusted by another, is of very little value in giving real knowledge. In order that the knowledge shall be made alive, it must be a part of the student's experience by actual experiments carried out by the student himself."

Beale, in his work on the microscope, expresses it thus: "The number of original workers emanating from our schools will vary as practical work is favored or discouraged. It is certain that they who are most fully conversant with elementary details, and most clever at demonstration, will be most successful in the consideration of the higher and more abstruse problems, and will feel a real love for their work which no mere superficial inquirer will experience. It is only by being thoroughly grounded in first principles, and well practised in mechanical operations, that any one can hope to achieve real success in the higher branches of scientific inquiry, or to detect the fallacy of certain so-called experiments."

And Hon. J. D. Cox, skilled alike in the arts of war, statesmanship, and science, in his notable address upon Systematic Instruction in the Microscope at the University, before the American Microscopical Society, in 1893, says: "I wish to urge the desirability of a somewhat extensive course of technical training in regard to the microscope. . . . Any one who desires to devote himself seriously to investigation with the microscope will find great advantage, as it seems to me, in devoting some time to the study of the instrument itself in all its parts, and the history of their development." The study of this whole address is urged upon the person interested in the just appreciation of the different parts of the microscope and their successful employment or improvement.

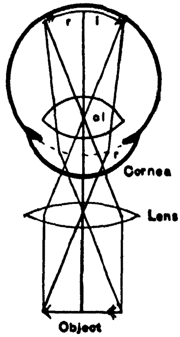


FIG. 1. A SIMPLE MICROSCOPE HELPING THE EYE TO FORM A RETINAL IMAGE OF A NEAR OBJECT.

Object The object to be seen by the eye.

Lens The double convex lens acting as a magnifier or simple microscope to aid the eye in seeing a near object.

Cornea The cornea of the eye.

r The single refracting surface in the schematic eye.

cl The crystalline lens of the eye, also the center of the refracting surfaces or the nodal point of the eye where the secondary axial rays cross.

ri Retinal image; it is inverted.

Sir A. E. Wright, in his book "Principles of Microscopy," says this: "Every one who has to use the microscope must decide for himself the question as to whether he will do so in accordance with a system of rule of thumb, or whether he will seek to supersede this by a system of reasoned action based upon a study of his instrument and a consideration of the scientific principles of microscopical technique. The present textbook [his "Principles of Microscopy"] has no message to those who are content to follow a system of rule of thumb, and to eke this out by blind trial and error. It addresses itself to those who are dissatisfied with the results thus obtained and who desire to master the scientific principles of microscopy, even at the price of some intellectual effort."

From the observations made during the last fifty years I am confirmed in the belief that for attainment in study with the microscope, as in all other human endeavor, a person must pay for all he gets.

(3) In considering the microscope, it may be looked at as a machine composed of glass and brass complete in itself, or it may be

considered as an artificial aid to the eye, like a spectacle. When complete in itself it is properly called a projection microscope, for it produces an image wholly independent of the eye of the observer. This image may be fixed on a photographic plate or used as a basis for a drawing (fig. 3). On the other hand, when used as a microscope in the ordinary way, the eye of the observer is an integral part of the optical combination, just as integral a part as the objective or the ocular (figs. 1, 2). This being the case the optical perfection of the eye is as influencing on the final retinal image as the perfection of the other optical parts.

Quoting again from the preface of the third edition: " In considering the real greatness of the microscope and the truly splendid service it has rendered, the fact has not been lost sight of that the

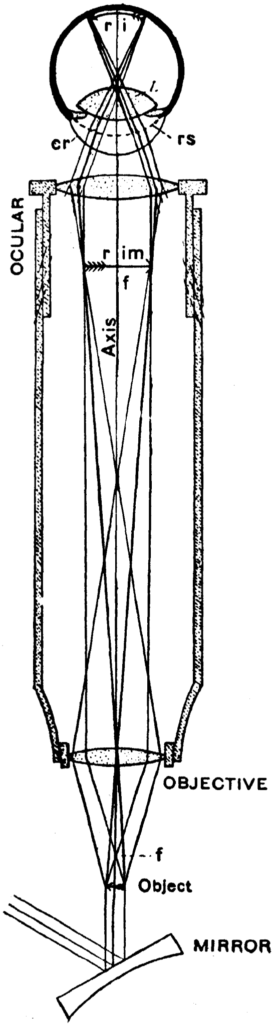


FIG. 2. A COMPOUND MICROSCOPE HELPING THE EYE TO FORM A RETINAL IMAGE OF A NEAR OBJECT.

Mirror	The plane and concave mirror to reflect light through the object.
Object	The small object to be seen by the eye.
Objective	The objective of the compound microscope to form a real image of the small object.
Axis	The principal optic axis of the microscope.
f	Principal focus of the ocular and of the objective.
r im	The real image formed by the objective.
Ocular	The double convex lens enabling the eye to see the real image formed by the objective.
cr	The cornea of the eye.
rs	The refracting surface of the schematic eye.
L	The crystalline lens of the eye.
r i	The retinal image; it is erect with reference to the object, but inverted as compared with the real image.

microscope is, after all, only an aid to the eye of the observer, only a means of getting a larger image on the retina than would be possible without it, but the appreciation of this retinal image, whether it is made with or without the aid of a microscope, must always depend upon the character and training of the seeing and appreciating brain behind the eye. The microscope simply aids the eye in furnishing raw material, so to speak, for the brain to work upon."

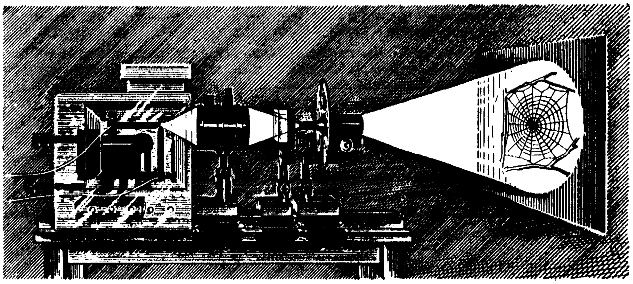
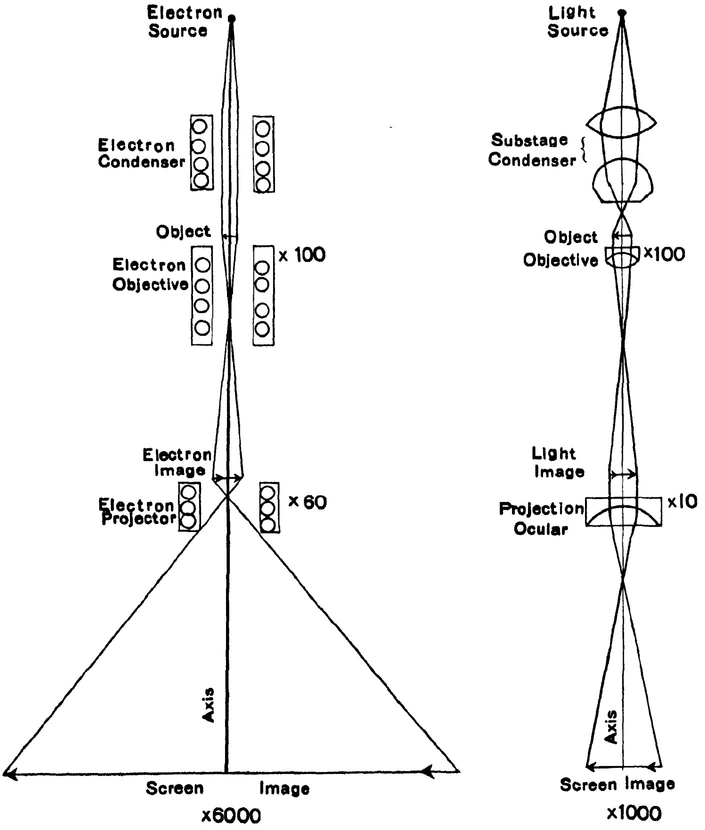


FIG. 3. PROJECTION MICROSCOPE WITH ENLARGED REAL IMAGE ON THE SCREEN

(4) While the objective and ocular are the fundamental constituents of a microscope, it must never be forgotten that for their most effective use provision must be made for so lighting the objects to be studied that their structural features may be brought out. This involves the use of a substage condenser to insure an adequate aperture of the illuminating light. This again necessitates a sufficient source of light either natural or artificial. If it is artificial, it must be sufficiently brilliant on the one hand, and on the other it is a great advantage to have it of daylight quality, such as that given by the Chalet Lamp with its daylight glass screens (figs. 46, 47).

(5) For gaining glimpses of structure and physical condition beyond what can be gained by the microscope lighted with ordinary visible radiation, either reflected or transmitted, one now has available dark-field illumination; illumination by polarized light, and radiation by the invisible ultra-violet. Perhaps also in the future,

radiation by the long waves of the infra-red may reveal structural details not hitherto known. In a word, to gain the deepest insight into microscopic structure every possible source of information should be utilized, and new ones constantly looked for.


COMPARISON OF

An Electron Microscope and A Projection Microscope

FIG. 3A. DIAGRAMS SHOWING THAT WHILE THE REAL IMAGE OF THE OBJECTIVES MAY BE EQUAL (x100 IN THIS CASE), THE SCREEN IMAGE CAN BE MUCH GREATER WITH THE ELECTRON MICROSCOPE.

ELECTRON MICROSCOPE

THE MICROSCOPE with glass lenses or glass lenses combined with lenses of natural transparent minerals like fluorite or quartz may be so constructed as to give almost any degree of magnification and thus make minute objects visible to the eye. However, for the showing of details as separate things, that is, for resolution, such a microscope is limited by the length of the waves of light to a magnification of about 1500 diameters.

Hence investigators, knowing that details beyond the ability of the ordinary microscope to reveal are probably even more pervasive than those visible with the best microscopes, have sought radiations of shorter wave lengths than those of visible light, hoping thereby not only to add to the magnification but to produce the corresponding increase in resolution.

It is believed that in the newly-invented electron microscope using electronic waves 1/100,000th of the wave-length of visible light, 25,000 to 100,000 magnification with accompanying resolution can be attained. This, of course, would make possible an almost unbelievable exploration of minute objects with their details, and it may be added, corresponding difficulties in interpreting the appearances.

The possibility of making such a microscope depends upon the fact that the electronic waves are propagated in straight lines like light waves and that they may be concentrated and focused by electromagnets something as light is concentrated and focused by glass lenses. Also, depending on the strength of the current in the electromagnet, the variable concentration and focusing are comparable to the concentration and focusing in the light microscope by means of different curvatures of the glass lenses.

As the electronic waves are wholly invisible, their images must be made visible either by using a fluorescent screen or by means of a photographic plate. Therefore the electron microscope is to be compared with a projection microscope in which the image formed is entirely independent of the eye. It is not like the microscope into which one looks, for with this the eye of the observer forms a part of the optical train, and the final image is formed upon the retina of the eye.

In the accompanying diagrams the constituents of an electron and of a projection microscope are shown for comparison; an electron source in one and a light source in the other; for illuminating the object a magnetic condenser in one and a glass condenser in the other; for producing a real image of the object, a magnetic objective and a glass objective respectively; and finally a magnetic projector and a glass projection ocular to form the screen image in each case.

The size of the final screen image may differ greatly in the two instruments, but varies most with the electron microscope, in which it may be as great as x250 while with the light microscope it rarely exceeds x10 or x15.

In the diagram the projection microscope is represented as producing a screen image of x100x10 = x1000 and the electron microscope x100x60 = x6000; but, as has been said, their respective possibilities are as great as about x1500 for the light microscope and x25,000 for the electron microscope; and this magnification, in some cases at least, might be increased to x100,000 in printing the negative.

There are three other differences that should be mentioned: (1) The initial cost of the best ordinary microscope is in the hundreds while that of the electron microscope is in the thousands of dollars. (2) All parts of the ordinary microscope can be used in the air of any room while all the effective parts of an electron microscope must be in as complete a vacuum as possible. (3) With the ordinary microscope the object can be of considerable size and thickness, and mounted upon a glass slip in air or some transparent medium like glycerol or Canada balsam. With the electron microscope the object must be small, very thin and dry and mounted on a film of collodion, which in turn is supported by a perforated disc of platinum or a fine wire screen.

It will be seen from the above that at present the electron microscope is not available for ordinary biological study, but is to be welcomed for the uses to which it is adapted now and for the possibilities in its future development.

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CHAPTER I

MICROSCOPES AND THEIR PARTS

§§ 1 TO 66; FIGURES 4-41

MICROSCOPES

§1. **Definition of a microscope.** — As the word itself indicates, a microscope is an optical instrument with which one can see small things, often so small that the unaided eye could not see them at all. It is from two Greek words: μῑκρός — mikros, small, and σκοπεῖν — skopein, to see. The word was compounded and given a Latin form by Giovanni Faber of the Academy of the Lincei, as shown by a letter of his to Cesi, President of the Lyceum, dated April 13, 1625. Faber says: "As I also mention his [Galileo's] new occhiale to look at small things and call it Microscopium." Jour. Royal Microscopical Society, 1889, p. 578; Carpenter-Dallinger, p. 125. The microscope serves its purpose by increasing the visual angle. This may be done in two ways: (1) by means of one or more lenses used as a kind of spectacle by which the eye is enabled to form a sharp image on the retina when optically so close to the object that without the artificial aid a sharp image could not be produced (figs. 1, 2, 6).

(2) The second way of increasing the visual angle is by means of a projection microscope, which, wholly independent of the eye, produces a sharp, greatly enlarged image of the object upon a white surface or other screen. The eye then looks at this image as though it were the object itself and of that size (fig. 3, § 445).

The fundamental difference in the two forms of microscope is that in the first the image is formed in the eye by rays directly from the microscope, in the second by rays from the screen.

In this book the first form of microscope is mainly considered except in Ch. IX and X, where the projection microscope is much used.

SIMPLE AND COMPOUND MICROSCOPES

§ 2. **A simple microscope** or magnifier is a lens or a combination of lenses to use with the eye. But one image is formed and that is upon the retina. The enlarged image has all its parts in the same position as they are in the object itself, that is, the image appears exactly as with the naked eye, except that it is larger (figs. 5–6).

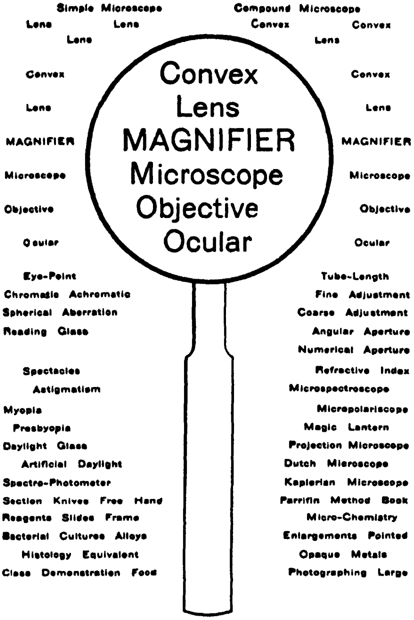


FIG. 4. FINE PRINT SEEN BY THE UNAIDED EYE AND THROUGH A MAGNIFIER

§ 3. **A compound microscope** is one in which a lens, or combination of lenses, called an objective, forms a real image, and this real image is looked at, by the eye and a magnifier, or ocular. The image seen has the object and its parts inverted. In the compound microscope then, two images are formed, one by the objective independent of the eye, and the other on the retina by the action of the eyelens of the ocular and the cornea and crystalline lens of the eye (fig. 1).

§ 4. **Real images.** — A real image is one formed by a lens or other optical instrument, like a concave mirror. It is called real because, entirely independent of the eye, it forms a picture of an object. This is the kind of image which makes photography possible, also the magic lantern, and moving pictures on a screen.

§ 5. **Virtual images.** — In all diagrammatic drawings showing the microscope when looking directly into it, an enlarged, imaginary object is shown out in space. This is frequently called a virtual image. In the projection microscope there is an actual or real, enlarged image on a screen which the observer looks at as if it were

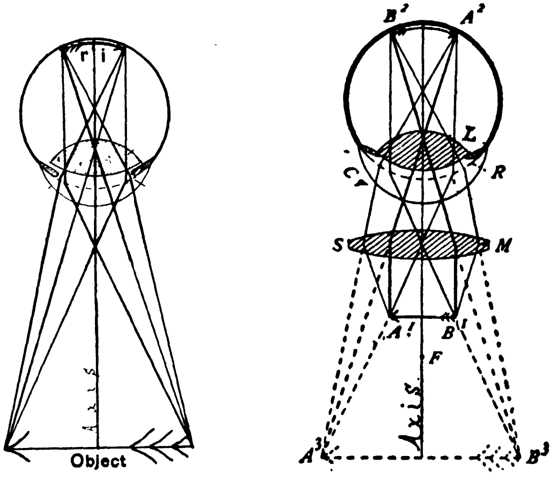


Fig. 5–6. Vision by the Unaided Eye and by the Aid of a Simple Microscope.

Fig. 5. Unaided Eye Vision. Axis, the Principal Optic Axis of the Eye Extended to the Object.

Object The object to be seen; it is at a distance of 250 millimeters from the eye.
r i The retinal image; it is inverted.

Fig. 6. Vision by the Aid of a Simple Microscope. Axis, Principal Optic Axis of the Microscope and of the Eye.

A^1 B^1 The object within the principal focus (F) of the lens.
S M A double convex lens acting as a simple microscope.
Cr The cornea of the eye.
R Single refracting surface of the schematic eye.
L The crystalline lens of the eye.
B^2 A^2 The retinal image; it is inverted.
A^3 B^3 The virtual image projected into the field of vision at 250 millimeters; it is erect, and the appearance is exactly as if the virtual image were an object as in fig. 4, and no lens were present.

a large object (fig. 3). If one keeps in mind that virtual images are purely imaginary, and that real images are produced by actual rays of light, it will help to avoid confusion and wrong interpretations.

In every case where an object is seen, light rays must pass from the object to the eye, and these rays entering the eye must form an image on the retina. It is the retinal image which furnishes the brain the stimulus for vision.

APPARENT SIZE OF OBJECTS

Whether one is using a microscope or not, the apparent size of any object seen depends upon the visual angle.

§ 6. **Visual angle.** — This is the angle made by the border rays of light from the object to the retina, and crossing at the nodal point or optical center of the eye (figs. 143-144).

As the visual angle depends upon the distance the object is separated from the eye, any means by which the object can be brought closer to the eye will result in giving a larger apparent size to the object, or in magnifying it. The lenses of the microscope used with the eye enable it to get very close to the object and thus increase the visual angle, and depending on the closeness, finer and finer details of the object are separated, for they subtend an angle of one minute or more (see § 359), and the object as a whole has a much greater apparent size. For further discussion see §§ 359-360.

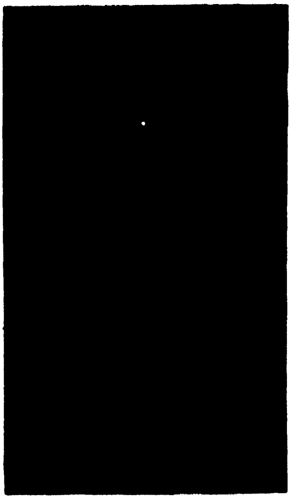


FIG. 7. PIN-HOLE CARD FOR VIEWING NEAR OBJECTS.

§ 7. **Pinhole card.** — Use a piece of paper about the size of a library card. If the slip is black or of a dark color it makes the experiment a little easier than when white paper is used. Make a hole in this with a needle (fig. 7). If now one holds the slip up close to the eye and gets the hole in the optic axis, the eye can see brilliantly lighted objects very clearly. If, to start with, the object is off about 1 meter, quite an extent of it can be seen, and it will appear small. Now go up closer and closer, and still the object is clearly seen, and constantly appears larger. The closer one gets the smaller is the visible field, but the larger will the parts seem to be. If the hole is quite small, one can get the object within 4 or 5 cm. of the eye and still see the image clearly, and see details which could not be seen at a greater distance.

As shown in the figures of the visual angle (fig. 144), the closer the eye gets to the object the greater will be the visual angle, hence details are shown which did not appear at a greater distance. One of the best methods of trying this experiment is to use for object a small mark made with ink or a glass pencil on a window or on a milky or transparent lamp shade. Then there will be plenty of light. The physiological explanation of the power to see clearly through the pinhole at a distance of 5 cm., when, if the eye looks directly at the object, it should be about 25 cm. from the eye, is that with the pinhole the beam is so narrow that the rays entering the eye are practically parallel. If one takes away the card, the beam gets very wide and the eye has only a blurred impression, the diffusion circles are so large.

In case one loses his spectacles or has the accommodation paralyzed by atropin for testing the eyes, it is possible to read fairly well with the perforated card if the print is in a brilliant light. The field which can be seen at one time is very small, so one must move the print or the head almost constantly.

Lenses

The usual and most effective means for increasing the visual angle when examining small objects is by the use of lenses, singly or in combination.

§ **8. Lens.** — A lens means a mass of glass or transparent mineral substance with one plane and one curved, or with two curved surfaces.

The lens is usually a segment of a sphere or of two spheres (fig. 8). In dealing with lenses mention must frequently be made of the optical center of the lens, the principal axis, secondary axis, and the principal focus. These are illustrated in figs. 8, 11–12, and are briefly:

(1) *Optical center.* — The point in or near a lens through which, if rays pass, they will suffer no angular deviation, and the emerging ray will be parallel to the incident ray (fig. 8 *c.l*).

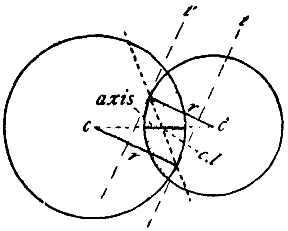


Fig. 8. Lens with Outlines of the Two Spheres of which it is a Segment.

Axis The principal optic axis, the line joining the two centers of curvature (*c c′*).

c c′ Centers of curvature, — centers of the two spheres from which the lens is derived.

r r′ Parallel radii.

t t′ Tangents at the terminal points of the radii.

cl Center of the lens, — point where the line joining the radii at the tangential points crosses the principal axis.

(2) *Principal axis.* — The axis passing through the centers of curvature of the two spheres whose surfaces bound the lens (fig. 8).

(3) *Secondary axis.* — Any axis oblique to the principal axis, but passing through the optical center of the lens (figs. 11–12). A ray along a secondary axis undergoes no angular deviation, although it may suffer displacement as a ray in traversing a piece of plane glass (fig. 99).

(4) *Principal focus.* — The point where rays of light, parallel to the principal axis, cross after traversing the lens (fig. 10). Every lens has two principal foci, one on each side (fig. 10.)

With concave lenses the foci are virtual (fig. 9).

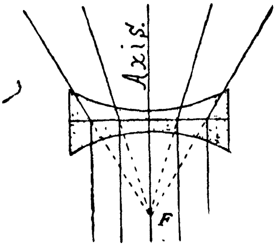


Fig. 9. Concave Lens Showing Virtual Focus (F).

§ 9. **Refraction.** — By this is meant the change of direction of oblique light in passing from one transparent medium into another of different density. The possibility of the production of images by lenses depends upon refraction. (See § 239 for non-oblique light and refraction.)

The amount of bending of the oblique rays depends on two things:

(1) The difference of density of the two refracting media; the greater the difference, the greater the refraction.

(2) The obliquity with which the light strikes the second medium.

The greater this obliquity, the greater the bending of the light, in accordance with the law of sines (§ 240).

§ **10. Geometrical construction of images.** — In this book the lenses shown are thick, but the course of the rays, for simplicity, is shown to be as if the lenses were infinitely thin, that is, they show all the bending at one plane (the refracting plane, figs. 11–12). In reality there is one refraction at the incident or entering surface and one at the emerging surface. With thick lenses like those figured, there will be no angular deviation for rays traversing the optical center of the lens, but there will be a certain amount of displacement, although the emerging ray will remain parallel to the entering or incident ray (fig. 64).

For the construction of images it is necessary to know the position of the principal focus and the optical center of the lens.

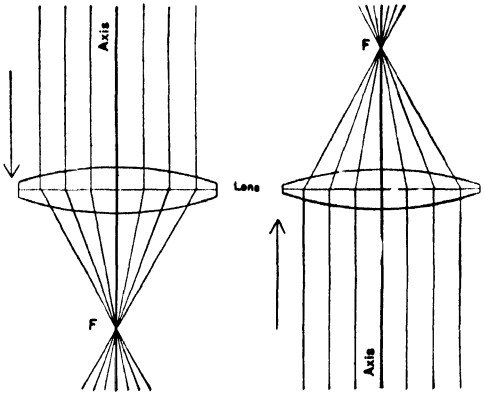


FIG. 10. LENS WITH A PRINCIPAL FOCUS ON EACH SIDE.

Axis The principal optic axis.

F The principal focus, — the point on the axis at which rays parallel with the principal axis cross.

The arrows indicate the direction of the light.

It should be remembered in making the drawings for the geometrical construction of images that there are two fundamental laws which must always be obeyed.

(1) Light rays extend in straight lines in a transparent medium of uniform density, and whenever the direction is to be changed the light must meet a different refracting medium, or a reflecting surface. That is, the direction of a ray of light may be changed by using a mirror, or by putting in its path a transparent medium of greater or less refracting power.

(2) The second law is, that objects are always seen in the direction in which the light reaches the eye, regardless of the actual position of the object. This will be abundantly illustrated in the chapter on drawing; and every one knows that objects seen in a mirror are not where they appear to be in the mirror.

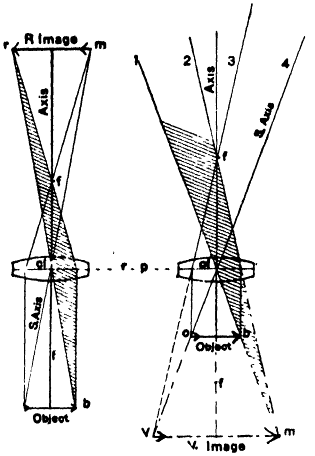


Fig. 11–12. Geometrical Construction of Real and of Virtual Images.

Object, *Object* The object of which an image is to be formed.

Axis, *Axis* The principal optic axis extended above and below the lens to the object and image.

S Axis, *S Axis* Secondary axis passing from the object through the center of the lens.

f, *f*, *f*, *f* The principal foci of the two lenses.

r–p The plane of refraction (the ideal plane at which all the refraction is made to occur in diagrams of thick lenses).

R. Image Real image.

V. Image Virtual image indicated by broken lines as it has no real existence.

o b, *r m* Rays of light indicated by lines passing from the extremities of the object to the extremities of the real image, which is inverted.

o b, *1 2*, *3 4*, *v m* Lines representing rays of light from the object passing in a diverging manner above the lens, and extended by broken lines below the lens to form a virtual image at their crossing points, *v m*.

§ 11. Construction of real images. — (1) The object must be situated outside or beyond the principal focal point (fig. 11).

(2) From some point in the object, draw a line to the refracting plane of the lens (§ 10) parallel to the principal axis, and from this crossing point at the refracting plane of the lens to the focus of the lens, and continue the line indefinitely (fig. 11).

(3) From the same point of the object as in (2), draw a secondary axis through the optical center of the lens and extend it indefinitely (fig. 11).

The image of the point in the object from which the two lines were drawn will be located at the point where the two extended lines cross above the lens (fig. 11).

The image of all the other points of the object may be determined by drawing lines from them exactly as just described.

If the image is known one can find the object by reversing the process just described.

§ 12. Construction of virtual images. — (1) For these the object must be somewhere between the principal focus and the lens.

(2) From some point in the object draw a line to the refracting plane of the lens, parallel to the

principal axis, and from this point through the principal focus, and continue it indefinitely.

(3) From the same point of the object as in (2) draw a secondary axis through the optical center of the lens and extend it indefinitely.

The two lines will not cross above the lens, but if they are extended below the lens (fig. 12) they will cross, and the crossing point locates the image. But as there are no light rays extending in this direction the image is imaginary or virtual. That is, it looks as if the rays reaching the eye originated from the point where the rays would cross if extended backward.

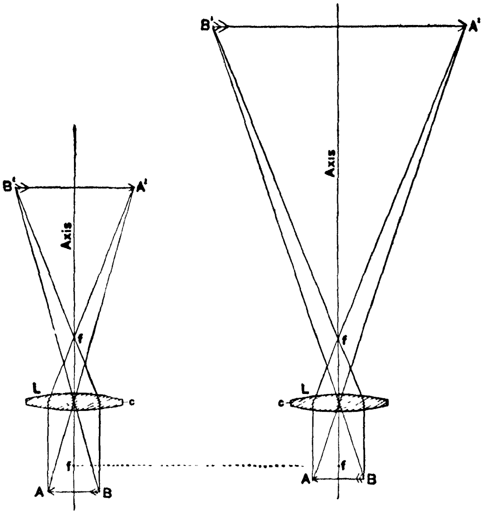


FIG. 13–14. REAL IMAGE WITH THE OBJECT FAR FROM AND NEAR TO THE PRINCIPAL FOCUS.

Axis, Axis The principal optic axis extended above and below the lenses.

f, f, f, f The principal foci of the lenses.

L c, L c The same lens with the object farther from and nearer to its principal focus.

A B, B' A' The object and its inverted image when the object is far from the principal focus.

A B, B' A' The object and larger inverted real image when the object is near the principal focus.

§ 13. Relative position of object and image. — The general law is that the nearer the object to the principal focus, the farther away is the image; and conversely, the nearer the image is to the principal focus, the farther from it must be the object. And from the law of similar triangles, the size of the image is to the size of the object as the distance of the image from the center of the lens is to the distance of the object from that center. In a word, the nearer the object to the principal focus the farther away the image from that point, and the greater the relative size of the image. This is equally true of real and of virtual images (figs. 13–14).

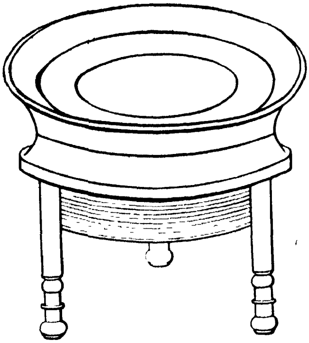


FIG. 15. TRIPOD MAGNIFIER.

§ 14. Mounting of simple microscopes. — Magnifiers are arranged in mountings to be held in the hand; for example, reading glasses and pocket magnifiers. The tripod magnifier (fig. 15) may be held in the hand or supported by its legs over the object to be seen. Sometimes there is a special support with arrangements for focusing as well as holding the magnifier in any desired position (fig. 17). This arrangement is especially desirable when magnifiers are used for dissection. For the purposes of dissection and examining objects under a small magnification, binocular arrangements like spectacles are very convenient, as one can move the head and bring the object into view at will.

COMPOUND MICROSCOPE AND PARTS

§ 15. Compound microscope. — This, as shown in figs. 2 and 18, and explained above, aids the eye in obtaining an enlarged retinal image by two steps, viz., the formation of a large real image by the objective and a retinal image of this real image by means of the microscope ocular, and the cornea and crystalline lens of the eye, the ocular acting in general like a simple microscope (§ 2).

For holding the objective and ocular and focusing the microscope, there are a number of mechanical arrangements necessary. For illuminating the object there is

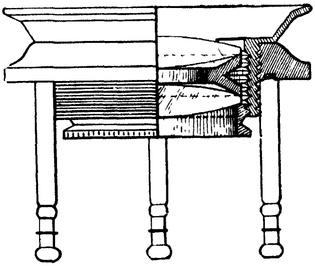


FIG. 16. TRIPOD MAGNIFIER WITH A SECTION REMOVED TO SHOW THE TWO COMPONENT, CONVEX LENSES AND INTERVENING DIAPHRAGM.

usually a mirror and often a condenser. It is customary and convenient to divide the parts of a compound microscope into two groups: (1) the optical parts, and (2) the mechanical parts (fig. 26).

OPTICAL PARTS OF A COMPOUND MICROSCOPE

§ **16. Objective.** — This is a lens, or combination of lenses, which, under the proper conditions, produces an enlarged, inverted image of some object (figs. 11, 18).

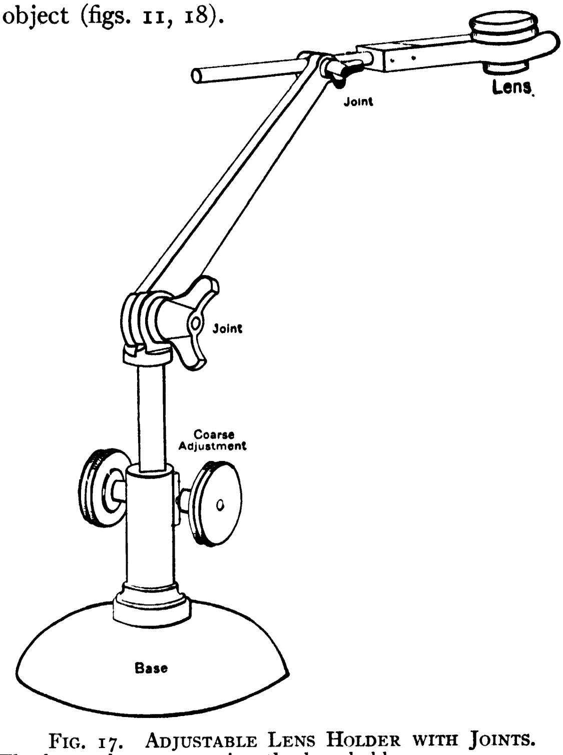


FIG. 17. ADJUSTABLE LENS HOLDER WITH JOINTS.
Base The heavy base supporting the lens holder.
Coarse Adjustment The rack and pinion for focusing the lens.
Joint, Joint The joints enabling one to put the lens in any desired position.
Lens This is held in a spring fork or in a socket.

Practically all microscopic objectives are composed of one or of several combinations of lenses. The purpose of combining the lenses is to produce an image as nearly as possible like the object itself, by doing away with certain defects or aberrations inherent in simple lenses (fig. 19–21).

§ **17. Optical designation of objectives.** — As will be seen in sections 20–34 objectives are designated in various ways to indicate one or more of their special qualities. They have also been merely lettered or numbered. This method is purely arbitrary, and gives no information.

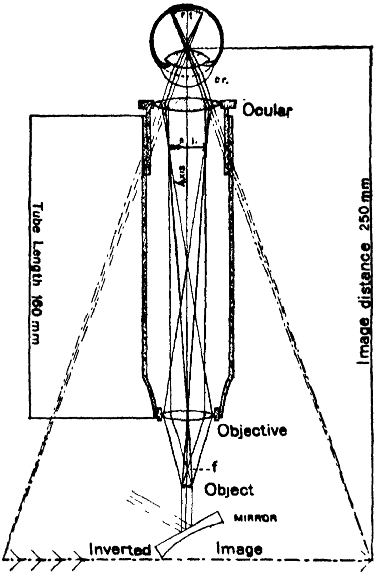


FIG. 18. DIAGRAM OF A KEPLERIAN, COMPOUND MICROSCOPE AND THE EYE OF THE OBSERVER TO SHOW THE DIFFERENT IMAGES, TUBE-LENGTH, AND THE IMAGE DISTANCE OF THE PROJECTED VIRTUAL IMAGE.

f Focal point of the objective.
r i Real image formed by the objective.
r t Retinal image in the eye.
cr Cornea of the eye.

In striving to find some method of designation which in itself would give some definite information to the user, microscope makers adopted the plan of engraving the equivalent *focal* length or focus (E.F.) upon each objective, thus indicating that at any given distance the objective composed of several lenses would give an image of the same size as a simple lens of the designated focal length.

The given distance agreed upon by practically all makers at which to measure the image is 10 inches or 250 millimeters, as this distance is assumed to be the one giving to normal adult human beings, the most perfect vision for near objects.

When the long-tubed microscopes were in use it was natural to adopt 10 inches or 250 millimeters for the tube-length, then the virtual image (fig. 18) would appear to be at about the level of the stage of the microscope where the actual object is situated, and the appearance to the observer is as if the object itself were of that size. From the optico-physiological standpoint this was a very logical tube-length to adopt; but with the study of living things and of objects in liquids it is almost imperative that the microscope be vertical and thus give a horizontal stage (fig. 26). With the microscope vertical, however, the top of the ocular was so high that the observer needed to use a very high chair for the ordinary table, or an especially low table. To overcome the difficulty, the tube of the microscope was shortened and is now almost universally 160 mm. from the level where the objective is inserted to the top of the tube. With the 250 mm. or 10-inch tube-length the special or initial magnification of the objectives and the oculars was found in the usual way by dividing 250 mm. or 10 inches by the equivalent focus (e.f.). For example, if the objective has an equivalent focus of 25 mm. (1 in.), its power would be rated as 250/25 = 10, or 10/1 = 10. With an ocular of the same equivalent focus the magnification would be found in the same way, and would likewise be 10. Then the total magnification of the microscope with a 25 mm. objective and a 25 mm. ocular would be 10x10 = 100. Working on this plan, it was necessary merely to mark the objective and ocular with their equivalent foci (e.f.), and the observer could make any desired combination.

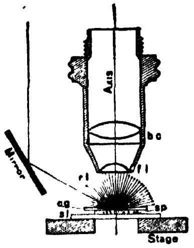
When the short or 160 mm. tube came into use, while the magnification of a given ocular remained as before, the magnification of the objective of any given e.f. was proportionally lessened by the shortened tube. For some time, however, the rating of the objective was not changed, and the total power of the microscope was made correct by giving the ocular a rating sufficiently below its actual power to compensate for the error introduced by shortening the tube. This was not a satisfactory solution, and finally in 1901–2 the Spencer Lens Company of Buffalo introduced the plan of giving the objectives their true magnifying power on the short tube, and the oculars their true rating; and instead of marking only the equivalent focus on the objectives and oculars, the initial magnification was added. For example, an objective of 16 mm. e.f. was given an initial magnification of 10x with the short tube, and the 25 mm. e.f. ocular was marked 10x also. With this combination the total magnification of the microscope would, then, be 10x10 = 100.

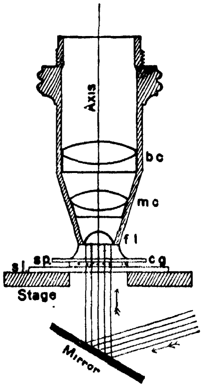
With this method of designation it is very simple to make any desired combination by selecting the initial magnifications of objective and ocular which when multiplied together give the power needed. It should be remarked, however, that one must not rely on this if exactness in a given case is necessary, for even when one makes the combination indicated there is considerable variation as one can see by consulting the table on page 67. In any special case one must determine the exact magnification of the combination used by one of the methods given in Chapter VIII.

§ 18. Determination of the initial magnification of an objective with the 160 mm. tube-length. — The tube-length is made exactly 160 mm. This is most easily and accurately determined by inserting into the open body and draw-tube of the microscope a slender rod precisely 160 mm. long. A block of wood or other flat object is held against the opening for the insertion of the objective to stop the rod at the right point. Then the draw-tube is adjusted until the upper end of the rod is just on the level with the top of the draw-tube. It should be noted that with some of the earlier microscopes the mark of 160 mm. on the draw-tube indicated the proper tube-length without the revolving nose-piece; hence, to get the proper 160 mm. tube if the nose-piece is used, the draw-tube must be pushed in as many millimeters as the nose-piece adds at the lower end. With the newer microscopes the 160 mm. mark on the draw-tube indicates the tube-length with the nose-piece in place. All of which emphasizes the need of making a special determination in each case if real accuracy is desired by the worker.

After the tube-length is made correct, the given objective whose initial magnification is to be found is screwed in place and a 10x ocular is put into the tube.

For object a stage micrometer in 1/10th and 1/100th mm. is placed on the stage and sharply focused. The 10x ocular is then removed and a 10x positive ocular with micrometer rulings in 1/10th mm. is inserted. The ocular micrometer is sharply focused by raising or lowering the eyelens or combination, and then, without changing the focus of the objective in the least, the draw-tube is pulled out or pushed in until the lines of the stage micrometer are perfectly sharp upon the ocular micrometer. Make the lines of the two micrometers parallel and see how many of the spaces in the ocular micrometer are required to measure one or more of the spaces on the stage micrometer.





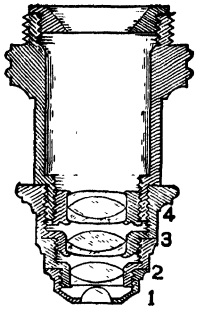


Fig. 19. Low Objective in Section.

Axis The principal optic axis of the objective.
fl The front lens of the objective.
bc The back combination composed of a concave and a convex lens.
Stage The stage of the microscope in section.
Mirror The mirror is above the stage in this case and reflects light down upon the object.
rl Reflected light from the object.
sl, *sp* The glass slide and the specimen on the slide.
cg Cover-glass over the specimen.

Fig. 20. High Power Objective in Section.

Axis The principal optic axis of the objective.
bc Back combination of a double convex and a plano-concave lens.
mc Middle lens combination.
fl Front lens of the objective.
cg, *sp*, *sl* The cover-glass, specimen, and slide.
Stage The stage of the microscope in section.
Mirror The mirror reflecting parallel rays up through the specimen.

Fig. 21. High-power Objective of Four Combinations.

1 The front lens.
2, *3*, *4* The three combinations of lenses, the back combination (*4*) composed of three lenses.

§ **19. Names applied to parts of objectives.** — As objectives have usually two or more combinations of lenses (figs. 19–21) it is convenient to have a name for each combination.

(1) Front combination. This is the part of the objective nearest the object.

(2) Back combination. The combination of lenses farthest above the object, and, hence, nearest the ocular.

(3) Intermediate or middle combination. The lenses between the front and back lenses. Sometimes there are two or more intermediate combinations (fig. 21).

KINDS OF OBJECTIVES

Depending on their construction or manner of use, objectives have received special designations or names.

§ **20. Dry objectives.** — These are objectives in which air is between the objective and the object or cover-glass (fig. 43).

§ **21. Immersion objectives.** — With these there is some liquid between the front of the objective and the object or the cover-glass (fig. 20). Immersion objectives are usually designated by the name of the liquid used.

§ **22. Water immersion objectives.** — With these there is water between the cover-glass or the object and the front lens.

§ **23. Homogeneous or oil immersion objectives.** — The immersion liquid in such objectives has the same refractive index (see § 269) as glass, hence the light suffers no refraction in passing from the glass slide and cover-glass into the immersing liquid, and from that into the objective. As the liquid used with these objectives is nearly always thickened cedar-wood oil, they are more frequently called oil immersion than homogeneous immersion objectives.

§ **24. Achromatic objectives.** — These are objectives in which the image is practically free from rainbow colors. They are composed of one or more combinations of convex and of concave lenses (see § 257, under chromatic aberration). All good microscope objectives are achromatic.

§ **25. Aplanatic objectives, etc.** — These are objectives or other pieces of optical apparatus (oculars, illuminators, etc.) in which the spherical distortion is wholly or nearly eliminated, and the curvatures are so made that the central and marginal parts of the objective focus rays at the same point or level. Such pieces of apparatus are usually achromatic also.

§ **26. Apochromatic objectives.** — By this is meant objectives in which by means of special forms of glass and a natural mineral (*calcium fluorid, fluorite, fluor-spar*) the color and the spherical corrections have been made especially perfect, that is, rays of three spectral colors are combined into one focus instead of rays of two colors as with the ordinary achromatic objectives.

§ **27. Fluorite objectives.** — These are objectives containing one or more fluorite lenses with lenses of the new kinds of glass. They are more perfect than the achromatic objectives, approximating the apochromatics, and are good for photography.

§ **28. Non-adjustable or unadjustable objectives.** — Objectives in which the lenses or lens systems are permanently fixed in their mounting so that their relative position always remains the same. Lower power objectives and those with homogeneous immersion are mostly non-adjustable. For beginners and those unskilled in manipulating adjustable objectives (§ 29), non-adjustable ones are more satisfactory, as the optician has put the lenses in such a position that the most satisfactory results may be obtained when the proper thickness of cover-glass and tube-length are employed.

§ **29. Adjustable objectives.** — An adjustable objective is one in which the distance between the systems of lenses (usually the front and the back systems) may be changed by the observer at pleasure. The object of this adjustment is to correct or compensate for the displacement of the rays of light produced by the mounting medium and the cover-glass after the rays have left the object. It is also to compensate for variations in tube-length (§ 149). As the displacement of the rays by the cover-glass is the most constant and important, these objectives are usually designated as having cover-glass adjustment or correction. (See also practical work with adjustable objectives, § 149).

§ **30. Variable objective.** — This is a low power objective of 36 mm.

(4x) to 26 mm. (6x) equivalent focus, depending upon the position of the combinations. By means of a screw collar the combinations may be separated or brought closer together. If they are separated the power is diminished; and if brought closer together, the power is increased.

§ 31. **Illuminating or vertical illuminating objectives.** — These are designed for the study of opaque objects with good reflecting surfaces, like the rulings on metal bars and broken or polished and etched surfaces of metals employed in micro-metallography. The light enters the side of the tube or objective and is reflected vertically downward through the objective and thereby is concentrated upon the object. The object reflects part of the light back into the microscope, thus enabling one to see a clear image.

§ 32. **Dark-field objectives.** — Objectives for the microscope constructed with a numerical aperture low enough so that no light from the dark-field condenser can enter the objective directly. For homogeneous immersion objectives this is accomplished by inserting a reducing diaphragm, or by a special construction or mounting of the objective. (See also § 183.)

§ 33. **Ultra-violet objectives.** — Objectives constructed of quartz or ultra-violet transmitting glass.

§ 34. **Low and high objectives.** — A low objective is one that magnifies relatively little, and a high objective is one that magnifies much (figs. 19–21). By looking for the equivalent focus or the initial magnification of an objective one can tell precisely concerning its magnification. The mounts of objectives are, for the most part, so nearly alike that it is not easy to tell them apart at a glance. It is possible so to mark them with different colored rings that no confusion need arise when one is deeply immersed in thinking about the structures being examined. The author has adopted the following color scheme: The colors chosen are those of the sun spectrum. The color of longest wave-length, red, is used for the objective of longest focus (40 or 32 mm.); for the 16 mm., pink; for the 8 mm., orange-yellow; for the 4 mm., green; for the 3 mm., blue; and for the oil immersion, black. Colors avoid this confusion or loss of time in trying to see the equivalent focus or the initial magnification.

Perhaps a better method would be for the manufacturers to make the mountings of the objectives so different that there could be no confusion.

Oculars and their Designation

§ **35.** An ocular or eyepiece for the microscope consists of one or more converging lenses or lens systems next the eye. Its main purpose is to act with the eye as a magnifier of the real image formed by the objective (fig. 18). Incidentally the ocular also serves to correct some of the defects of the objective.

Oculars may be divided into groups according to their construction or action.

§ **36. Positive oculars.** — With these the real image of the objective is formed below all the lenses of the ocular (figs. 22, 23) hence all the lenses of the ocular, together with the eye, form a real image on the retina, of the real image formed by the objective.

§ **37. Negative oculars.** — In these the real image formed by the objective is between the lenses (figs. 24, 25).

In a negative ocular the lower or field lens acts with the objective to form the real image, while the upper or eyelens acts with the eye to form a retinal image of the real image (figs. 24, 25).

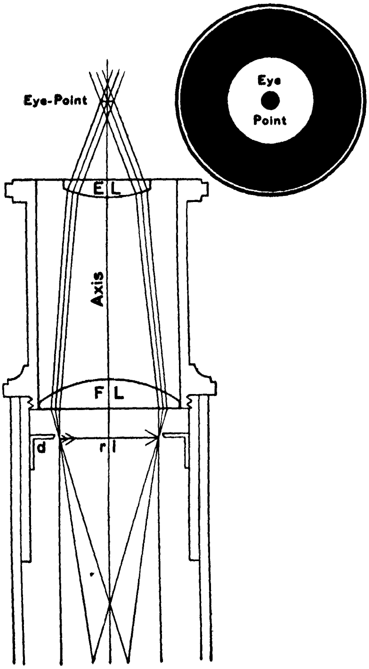


Fig. 22. Ramsden Ocular with the Real Image below and the Eyepoint above.

Axis The principal optic axis of the ocular.

d, ri The ocular diaphragm and the real image formed by the objective below all the lenses of the ocular.

Fl The field lens of the ocular.

El The eyelens.

Eyepoint The eyepoint in section and in face view, looking at the upper end of the ocular.

Positive and negative oculars can be readily distinguished by inspection, as the ocular diaphragm, at the level where the real image of the objective is formed, is between the lenses of the negative type, and below all the ocular lenses of the positive type (figs. 22, 23, 24).

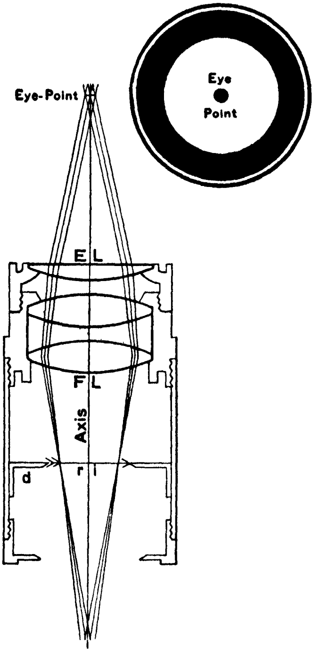
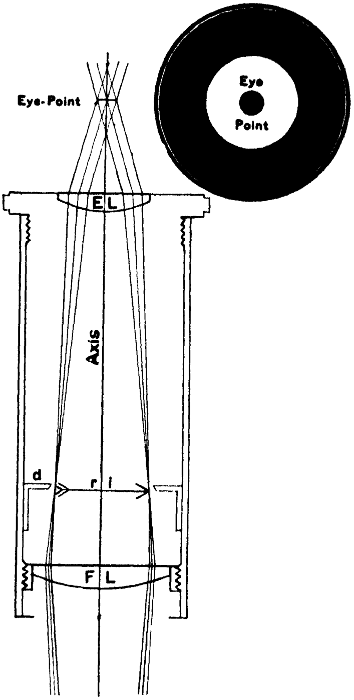


FIG 23. POSITIVE COMPENSATION OCULAR.



Axis The principal optic axis of the ocular.
d, ri The ocular diaphragm and the real image.
FL The field combination composed of three lenses.
EL The eyelens.
Eye-point The eyepoint in section and as seen by looking down upon the end of the ocular.

FIG. 24. LOW-POWER HUYGENIAN OCULAR IN SECTION.

Axis The principal optic axis of the ocular.
FL Field lens of the ocular.
d, ri Diaphragm and real image between the ocular lenses.
EL Eyelens of the ocular.
Eyepoint The eyepoint seen in section and by looking down upon the end of the ocular.

§ **38. Huygenian ocular.** — A negative ocular devised by the Dutch astronomer Huygens is the most common ocular used on the microscope, and consists of a plano-convex field lens and a similar but higher power, eyelens, the convex surfaces of both facing downward (figs. 24, 25). Theoretically the focal length of the field lens is about three times that of the eyelens, but in practice the ratio varies with the power, being 1 to 1.5 or 1 to 2 with low powers and nearer 1 to 3 with the high powers. The ocular diaphragm is placed approximately at the focus of the eyelens.

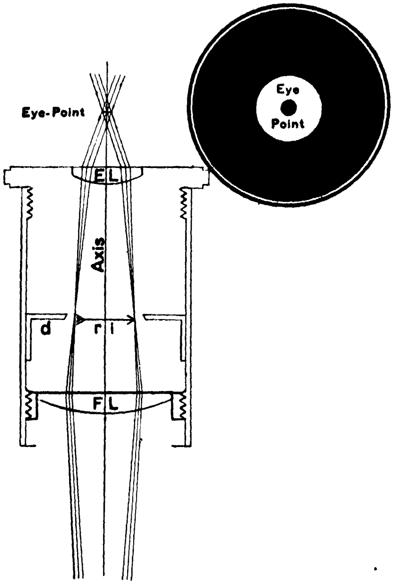


FIG. 25. HIGH-POWER HUYGENIAN OCULAR.

Axis The principal optic axis.
FL Field lens.
d, ri The diaphragm and real image between the ocular lenses.
EL Eyelens.
Eye-point The eye-point in section and face view, looking down upon the upper end of the ocular.

§ **39. Ramsden ocular.** — This is a positive ocular composed of two plano-convex lenses with the convex faces turned toward each other. and so arranged that the real image is formed below both lenses (fig. 22), not between them, as with the Huygenian ocular. In the best modern forms of Ramsden ocular the simple lenses are not used, but achromatic combinations. The Ramsden form is often used for ocular micrometers (§ 378).

§ **40. Compensating oculars.** — These are either positive or negative oculars chromatically overcorrected to compensate and correct the residual color defects in the extra-axial portion of the visual field due to the non-achromatic front lens of the objective (fig. 23). They are regularly used with apochromatic objectives, and may be used to advantage with high-angled objectives of the ordinary type. (See further, § 260.)

§ **41. Telaugic oculars.** — These, as the name indicates, have a high eyepoint making it possible for persons who wear spectacles for eye defects to keep the glasses on while looking into the microscope. Most oculars of the usual form have the eyepoint so close to the ocular that one cannot wear spectacles and get the eye close enough to the eyelens to see the entire microscopic field (§ 145). Besides the high eyepoint, these oculars give a large, flat, brilliant field. The 10x used by the author is positive, and is composed of two combinations with the convex faces inward, and the plane faces outward as in fig. 22.

§ **42. Projection oculars.** — These are oculars in which the upper combination of lenses is movable to enable one to focus the real image upon different distances of the receiving screen. They are especially useful in photographing with high powers (§ 474b).

§ **43. Ultra-violet oculars.** — Oculars whose lenses are composed of quartz or ultra-violet transmitting glass.

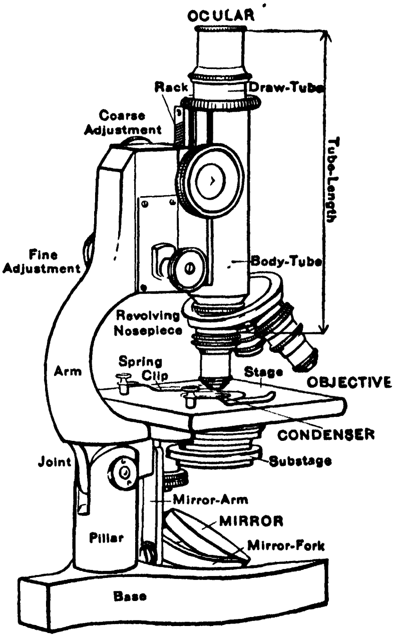


FIG. 26. LABORATORY COMPOUND MICROSCOPE WITH THE PARTS NAMED.

Mirror, Condenser, Objective, Ocular The optical parts of the microscope.

Tube-length This is the space between the insertion of the objective below and that of the ocular above. It is most commonly 160 millimeters.

Mechanical parts These are named in order from the base.

§ **43a. Trade names for oculars.** — These are very numerous as: holoscopic, hyperplane, orthoscopic, planoscopic, etc. In these the simple Huygenian and Ramsden forms are usually somewhat modified with the purpose of improving the optical qualities.

So-called demonstration oculars are also available by which two persons can look into the same microscope at once. This recalls the devices of Harting and Nachet by which two, three or four persons could look into the same microscope. (Harting, vol. 3, 1866, figs. 120–127).

§ **44. Designation of oculars.** — Formerly, oculars were, and to some extent at present still are, lettered or numbered A, B, C, D; 1, 2, 3, 4; I, II, III, IV; etc. This is a purely arbitrary designation except that the earlier the letter or the lower the number, the lower the magnification.

At present the progressive manufacturers give the equivalent focus or the initial magnification or both. If the magnification is given, it shows how much the ocular increases the magnification of the objective. If the power of the objective is given (§ 18), one can get the magnification of the combination approximately correct by multiplying the power of the objective by the power of the ocular, but see the table on page 67. However, the exact magnification in any given case must be especially determined (§§ 367, 396).

BINOCULAR MICROSCOPES

Very early in the history of the telescope and of the compound microscope, as nature has endowed us with two eyes, it was insisted upon that both eyes should be used in examining objects instead of using only one eye. This required two similar microscopes or telescopes side by side and the right distance apart for the two eyes. There still persists in the common opera-glasses the original binocular Dutch telescope-microscope.

§ **45. Binocular microscopes with two objectives and two oculars.** — These are in principle like the original binocular microscope of Cherubin d'Orleans (1677) except that his had no erecting prisms.

These instruments have been greatly improved in every way, and with the devices for quickly changing the paired objectives are indispensable in a biological laboratory, especially where much dissection under the microscope must be undertaken, and where objects are to be seen in relief, like the villi of the intestine, etc. The mountings of these binoculars are as varied as the uses to which they are put. One of the simple forms of binocular dissecting microscopes is shown here (fig. 27). The reader is advised to secure catalogues of the manufacturers in which are illustrated all the different forms. He can then select the one best adapted to his purpose.

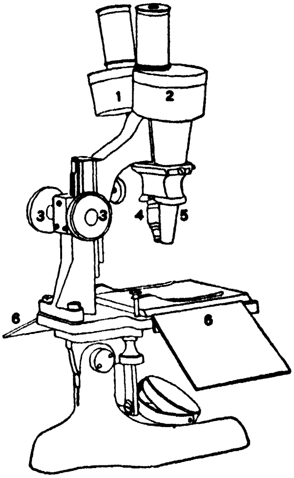


FIG. 27. DOUBLE OBJECTIVE BINOCULAR OF THE GREENOUGH TYPE

(Outline drawing based on Microscope KA, of the Bausch & Lomb Optical Co.)

1–2 The drums containing the Porro erecting prisms. These may be rotated to give the correct interpupillary position to the oculars.

3 Focusing wheels.

4–5 The two similar objectives. *4* is fitted with a fine adjustment to compensate for difference of focus of the eyes of the observer.

6 Hand-rests when dissecting an object on the stage.

§ **46. Binocular microscopes with two oculars, but with a single objective.** — The double microscope with two complete tubes, two objectives, and two oculars is not available for high powers, for the two objectives cannot be close enough together to bring an exceedingly small object into the field of both microscopes at the same time. Naturally, therefore, an effort was made to use a single objective and to divide the light passing through it so that half should go to the right and half to the left eye. The first successful binocular of this kind was invented by Riddell of New Orleans in America in 1851. In this, four prisms are used just above the objective which serve to divide the light equally and to pass it on to the two eyes through two parallel tubes, each with its own ocular. Later a satisfactory form was invented by Mr. Wenham of England in which there is but a single prism (fig. 28). Neither of these forms permitted of very high powers.

The light from two sides of the objective was separated and sent to the two eyes just as if the single objective were divided into a right and left half. This gave to each eye half the aperture and half the diffracted light from any one point, and therefore gave only the resolution, brilliancy and clearness of image of half the aperture. It was believed, however, that since the two eyes receive all the aperture, the brain in fusing the two images would give the impression that would be received by one eye receiving the full aperture, and besides would give a stereoscopic effect. These binoculars were rather large and cumbersome, and were not much used for serious investigation.

§ **47. Necessary qualities of binocular microscopes** (§§ **45–46**). — 1. The light to each eye should be of the same color and of the same intensity.

2. The real image formed by the objective in each tube must be of the same size, then similar oculars can be employed (§ 49).

3. The full aperture and equal diffracted light should be supplied to each eye.

4. The ocular tubes should be laterally adjustable so that the eyepoints of the oculars may correspond with the pupillary separation in the eyes of the observer.

5. The entire microscope should be focused by a coarse and a fine adjustment as with monocular microscopes.

6. There should be a special focusing device on one side to compensate for slight differences in the two eyes.

7. For single-objective binoculars, objectives of all powers should give good results.

§ **48. Advantages and disadvantages of binocular microscopes.** — The advantage of using both eyes is unmistakable. Both are trained and stimulated alike as in naked-eye vision. The advice is common to keep both eyes open and to use the eyes alternately with the monocular microscope, but this advice is easier to give than to follow. As it is easier to see with the naked eye, the eye outside the microscope is likely to dominate the situation and the microscopic image disappears, consequently most users of the microscope shut one eye when looking into the instrument. It is easy to keep both eyes open by means of the screen or shade close to the unused eye (fig. 36); but the experience of many people who have used the monocular microscope during many years has been that the eye most used gains in ability to see fine details, but loses in sensitiveness to light. This is easily demonstrated by using a high power on

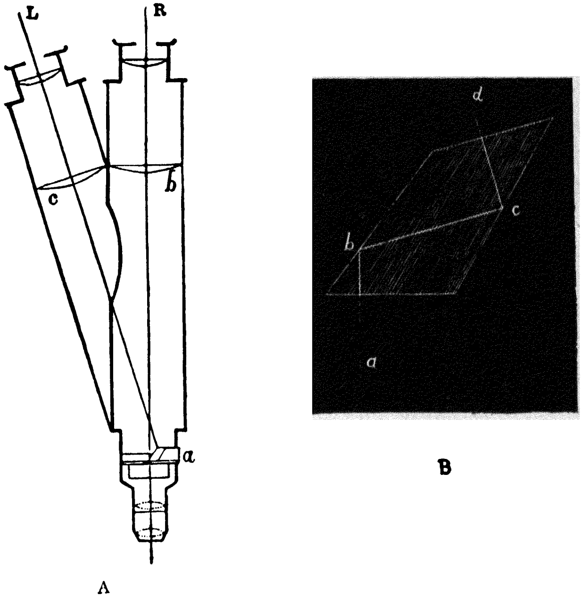


FIG. 28. WENHAM'S BINOCULAR MICROSCOPE.
(From Carpenter).

A Section of the microscope with the two converging tubes. By pulling out draw-tubes the oculars are separated for the correct pupillary distance of each observer.

L R The axes of the left and right tubes.
a The prism which divides the light from the object.
c b The field lenses of the two oculars.
B *Enlargement* of the dividing prism.
a, *b*, *c*, *d* Path of the light in the prism for the left eye.

As shown, the light to the right eye extends straight upward. This arrangement is limited to rather low powers.

fine details, and looking first with one eye and then with the other. No matter how sharply the image is focused, the " microscopic eye " can see the most detail, but the other eye sees a brighter image, but less detail. No doubt beginners can get the sense of depth, that is, the stereoscopic effect, more easily with a binocular than with a monocular. For those who have learned to judge of the relative vertical position or depth of objects by focusing up and down, no great help is given by the binocular, and even with the binocular the final test of relative depth must be by focusing up and down.

Among the disadvantages of the binocular may be mentioned its greater cost, and for many at least, the increased light necessary for illumination. The tube-length must be varied in lateral adjustment for the pupillary separation of different observers, and this changes the magnification which may interfere with the optical corrections (§ 143). For micrometry, photography, the micro-spectroscope and micro-polariscope it is not so well adapted as is a monocular instrument. To overcome these difficulties the manufacturers have provided a device for pushing the prisms aside and thus making a monocular instrument, or the entire binocular tube arrangement is removed and a monocular tube put in place — that is, in all modern forms provision is made for converting the binocular microscope into a monocular one (figs. 35, 36). Several manufacturers now produce binocular eye pieces for monocular microscopes.

While it is a great advantage to use both eyes in vision, as far as the microscope is concerned this is largely outweighed by the weariness that comes from holding the head so rigidly to keep the eyes over the eyepoints of the two oculars. In some cases workers in industrial plants have asked to go back to monoculars on account of the tiring effect of the rigid position. Of course, the advantage of using both eyes as in natural vision is gained by using some form of projection microscope like the Euscope described under drawing and demonstration (§ 444). Finally, it should be remembered that the more unlike the two eyes are, the less the usability of binocular instruments other than correcting spectacles.

§ **49. Modern single-objective binocular microscopes.** — In 1902 Mr. Frederick E. Ives, in a paper before the Franklin Institute of Philadelphia, showed how it was possible to construct a binocular microscope using one objective in which each eye received the full aperture from each point of the object and also shared equally the diffracted light. This microscope could be used for all powers from the lowest dry to the highest immersion objective. At that time he had constructed and used such a microscope (fig. 29). Instead of dividing the light reaching the objective into two halves, each half with half the aperture, he utilized a half-silvered prism which allowed half the entire light of every beam to pass through the tube to one eye, and reflected half to the other eye. In this way each

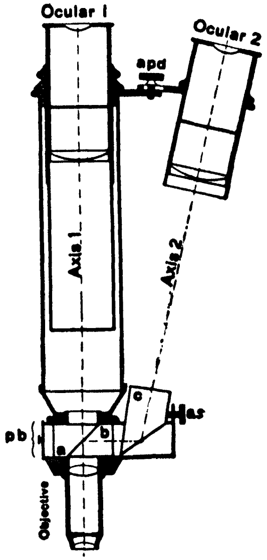


FIG. 29. IVES BINOCULAR ARRANGEMENT FOR ALL POWERS.
(Journal of the Franklin Institute, Dec. 1902).

Objective The single objective.
pb The prism box at the lower end of the tube.
a, b, c The prisms dividing the light equally from each point to the two eyes.
a, b The transparent silvered surface in the prism allowing half the light to pass through and half to be reflected to the right.
c Prism at the right reflecting the light upward to the right eye; *as*, adjusting screw to tilt the prism *c*, at the correct angle for the position of the right ocular.
apd Adjustment for the pupillary distance.
Ocular 1, Ocular 2 The oculars for the right and the left eye.
Axis 1 The principal optic axis for the left eye.
Axis 2 The principal optic axis for the right eye.
Due to the length of the prism *c*, this axis is optically of the same length as Axis 1 for the left eye.

eye receives the full aperture of light from each point of the object and also an equal share of the diffracted light. Furthermore, he showed that by a proper extension of the glass in the reflecting prism of the second tube the two optical paths were made equal, hence gave equal magnifications, and similar oculars were used for each tube, (fig. 29, Axis 1, Axis 2.)

Every point in which the new forms of binoculars are superior in optical performance over the original forms of Riddle and Wenham was clearly stated by Ives, except that now the half-silvered prism is half-coated with platinum instead of with silver. The platinum gives a more equal color to the two images.

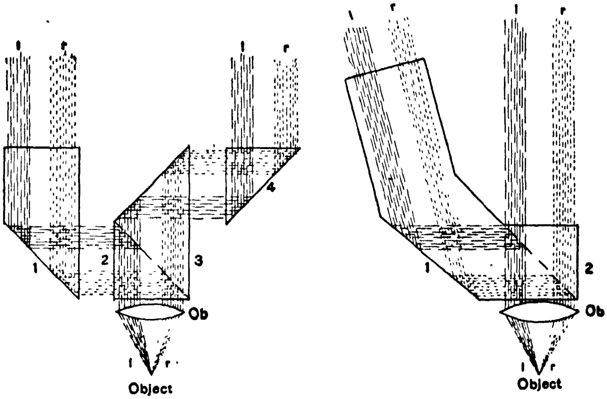
It is evident that the actual path in millimeters is greater for Axis 2 of fig. 29 than for Axis 1. This would result in the magnification being greater in the tube with the longer axis but for the optical device of extending the glass prism on that side sufficiently to elevate the position where the real image is formed without increasing the magnification. As in fig. 52 A, B, C the object seems to be raised by the thickening of the cover-glass, so extending the glass prism in the binocular raises the real image, and when the right thickness is used, the two tubes of the microscope are made optically equal. The practical opticians speak of this as a shortening of the optical path by means of the extra thickening of the glass prism (figs. 29-31). For Mr. Ives' original paper see the Journal of the Franklin Institute, vol. 154, Dec. 1902, pp. 441-445. See also Conrad Beck, Jour. Roy. Micr. Soc., 1914, pp. 17-23.

§ 50. Converging or parallel tubes for binocular microscopes. — The first binocular microscope (fig. 289) and all erecting binoculars at present have converging tubes. This is mechanically necessary to bring the two objectives close enough together and to separate the oculars sufficiently for the two eyes.

The single-objective binoculars of Riddle, Harting and Nachet had parallel tubes. That of Wenham had converging tubes. The modern forms are also divided on the arrangement of the two tubes. That of Ives had converging tubes (fig. 29), and those of the English opticians and of one American firm are also converging. The Continental opticians and one American firm have the tubes parallel.

The adherents of the converging form urge that as naturally the eyes converge for distinct vision at the near point, the tubes should converge accordingly. Those who use the parallel tubes urge that in microscopic work the eyes should be at rest as for viewing distant objects and therefore that the eye axes should be parallel.

In considerable experience with students and with others not especially familiar with optical instruments, it was found that with the converging tubes the binocular effect was more easily obtained than with the parallel ones. For some, however, the effect was quickly and easily gained with either form indifferently. Most observers can learn to use either form. Occasionally a person can never get the binocular effect with the parallel tubes, and not very satisfactorily with the converging ones.



FIGS. 30–31. PRISM ARRANGEMENT FOR TWO FORMS OF BINOCULARS FOR ALL POWERS.

(Conrad Beck, Jour. Roy. Micr. Soc., 1914.)

In Fig. 30 the arrangement is for parallel tubes, and in fig. 31 for converging tubes.

Object The object.

Ob The objective.

l, r; l, r The right and left beams of light emanating from the same point of the object.

As these beams extend through the objective and into the prisms they are equally divided so that half the right beam goes to the left and half to the right eye, and so with the left beam. This is indicated by the heavy and light broken lines by which the two beams are indicated.

1, 2, 3, 4; 1, 2 The four prisms in fig. 30, and the two prisms in fig. 31. The prisms are of the necessary length to make the optical path of the light equal for the two tubes, hence the magnification is equal for the two eyes.

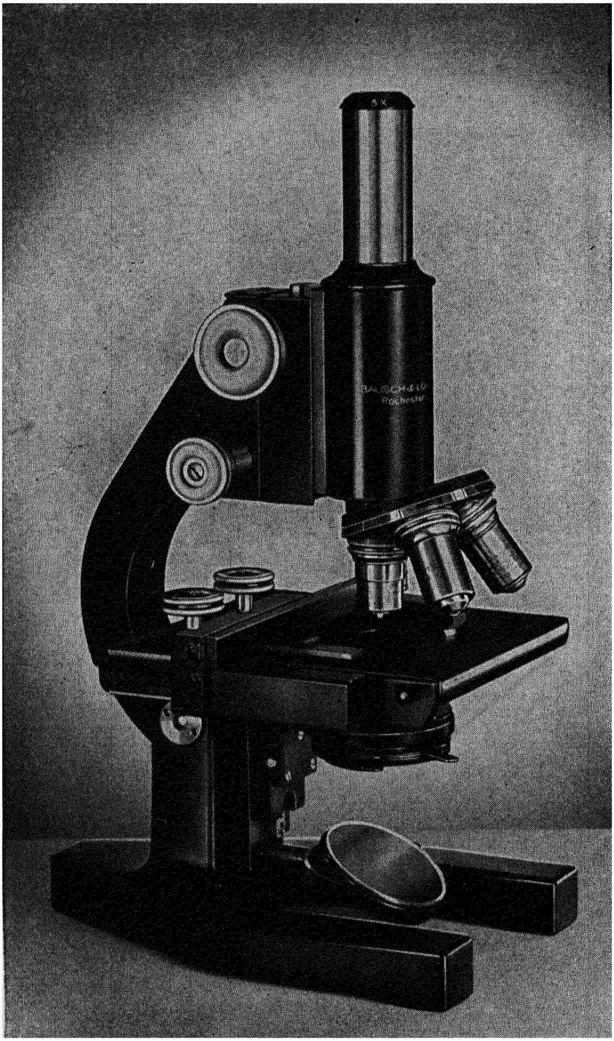


FIG. 32. THE BAUSCH & LOMB OPTICAL CO.'S MODEL HA-8 WITH MECHANICAL STAGE FOR STUDENT LABORATORIES.

(Courtesy of the Bausch & Lomb Optical Co.)

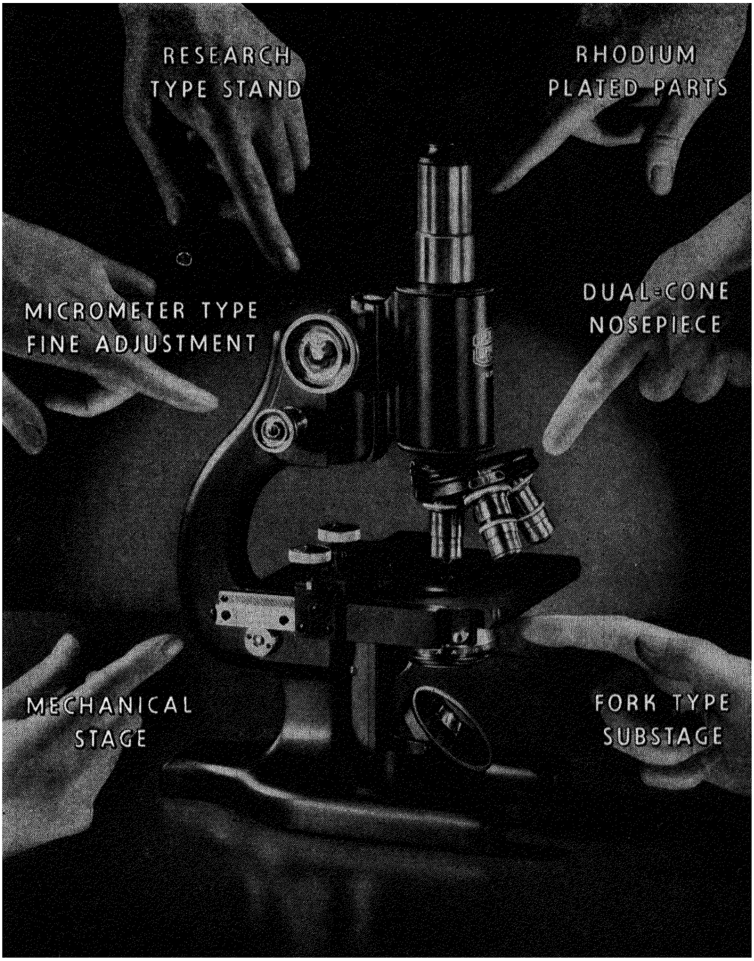


FIG. 33. THE SPENCER LENS CO.'S MODEL 13 MAH WITH MECHANICAL STAGE FOR STUDENT LABORATORIES.

(Courtesy of the Spencer Lens Co.)

CHARACTER OF COMPOUND MICROSCOPES

§ **51. Student microscopes.** — A great deal of beginning work with the microscope can be done with relatively simple and inexpensive apparatus. Fortunately, the manufacturers now furnish all their microscopes with excellent objectives and oculars so that the achromatic objectives and Huygenian oculars on their cheapest instruments are of the same quality as those with the more expensive outfits.

For student laboratories in colleges, medical and technical schools, junior colleges, and the more advanced preparatory and high schools the microscope should have at least the character and parts shown in figure 26. Such microscopes are now (1941) supplied by practically all the great microscope manufacturers. Examples of two American-made ones are illustrated in figures 32–33.

§ **52. Microscope stand.** — As seen in the figures 32–33, the mechanical parts are rugged. This renders them stable and gives the durability called for by the hard usage they will be likely to meet in actual laboratory use by beginners.

There should be a triple or a quadruple nose-piece with parfocalized objectives. A mechanical stage is often convenient.

§ **53. Optical equipment for laboratory microscopes.** — (1) There should be a plane and a concave mirror.

(2) There should be a substage condenser of 1.20 to 1.25 N.A. with iris diaphragm. The top element should be removable; then the lower element alone can light the whole field of the lowest objective. Furthermore, if the top is removable, a dark-field element can be put in its place. This will serve for much dark-field work. (See § 181.)

(3) There should be three achromatic objectives: (a) a 16 mm. divisible. When the front combination is removed or turned aside the upper combination forms a serviceable 32 mm. objective; (b) a 4 mm. objective, and (c) an oil-immersion objective.

(4) There should be two Huygenian oculars, one of 5x or 6.4x and one of 10x.

§ **54. Royal Microscopical Society standards.** — Down to 1857 and even much later with many microscope manufacturers, each one had a different screw for his objectives and a special size for oculars and substage condensers, tube-length, etc. In the year 1857 the Royal Microscopical Society of London urged standardization and began in earnest by designing a standard screw for objectives. Later, in 1896 and 1899, the Society again worked on standards, perfecting the "Society Screw" and recommending standard sizes for oculars and substage condensers. It is a great advantage to have all the parts standardized, then one can use on the same microscope stand the optics of any manufacturer. (See Beck, 1938 ed., pp. 18–19.)

§ **55. Pointer in the ocular.** — This is a slender rod of some sort situated at the level of the real image in the microscope, and it appears with the specimen in the field of view (fig. 34).

A pointer may be inserted in any Huygenian ocular as follows:

Remove the eyelens and with a little mucilage or Canada balsam, fasten a hair from a camel's-hair or other fine brush to the upper surface of the ocular diaphragm, and let it project toward the center of the free opening. The pointer will then appear in the field with the image, and by moving the specimen or rotating the ocular any particular structure can be pointed out just as one indicates the part of a magic lantern image on the screen or on a chart.

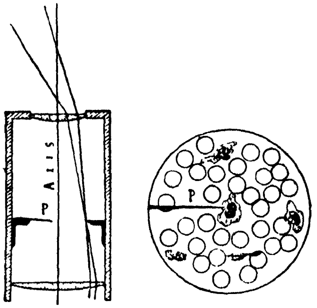


FIG. 34. POINTER OCULAR AND MICROSCOPIC FIELD.

P P The pointer attached to the diaphragm of the ocular and extending out into the free space.

At the right a field of blood corpuscles with the pointer indicating the position of a leucocyte.

If one uses positive oculars, a pointer can be put in the same way upon the top of the ocular diaphragm.

The ocular pointer was devised by Quekett. His was movable and was made of steel. It was called an indicator and the whole ocular was called an index eyepiece (Carpenter-Dallinger, p. 381; Carpenter, 6th ed., 1881, p. 112).

§ **56. Research microscopes (figs. 35–36).** — For over fifty years our country has been especially strong in the manufacture of the best stands and objectives, and during the last twenty-five years our

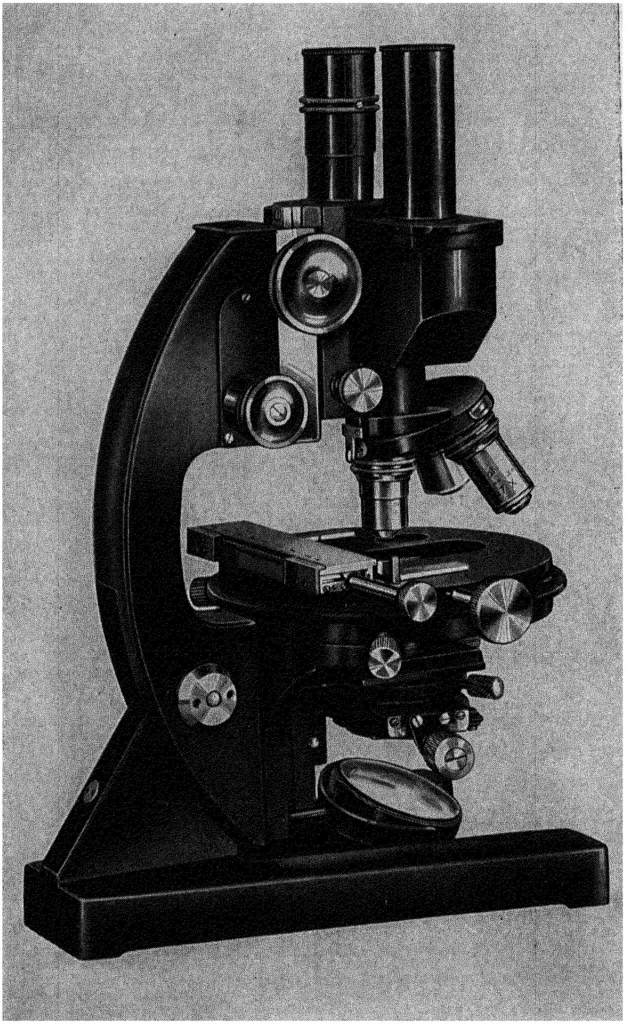


FIG. 35. THE BAUSCH & LOMB OPTICAL CO.'S MODEL GGDE RESEARCH MICROSCOPE WITH CIRCULAR REVOLVING AND MECHANICAL STAGE.

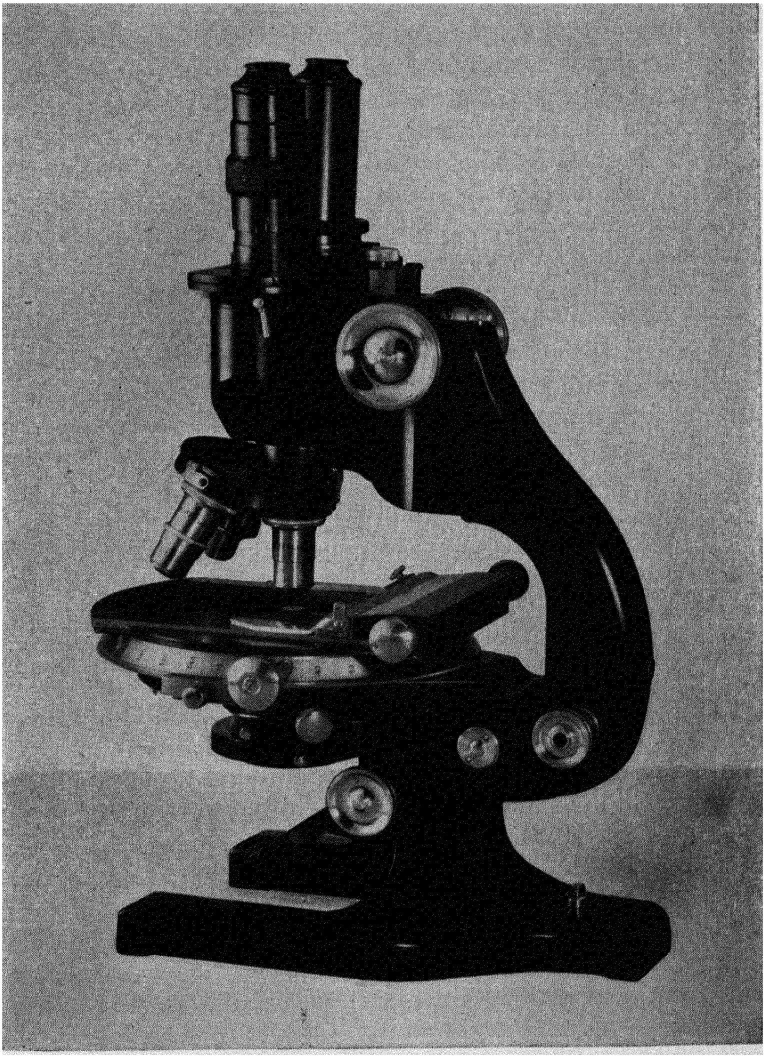


FIG. 36. THE SPENCER LENS CO.'S MODEL 3B WITH CIRCULAR REVOLVING AND MECHANICAL STAGE.

microscope makers have exerted all their skill to meet the demands from the biologist, the physiologist and the workers in the chemico-physical sciences. Fortunately, now almost any required arrangement of the mechanical parts and range in the optical parts can be obtained from American manufacturers, whose products are of the highest quality. Fortunately also, optical glass of the widest range is now produced here in any desired quantity. Furthermore, the microscope makers are ready and anxious to make apparatus of all kinds which shall meet a real demand.

While it is true that most of the fundamental discoveries that have been made by the aid of the microscope have been made with relatively simple apparatus, still for ease and certainty of accomplishment the more elaborate and precise modern instruments are desirable. But after all, it must be held in mind that it is the brain of the observer and not the elaborate apparatus that determines the final outcome of research.

When one is ready to buy a microscope, it is wise to get the latest illustrated catalogues of the various makers and select the form within one's means which seems best adapted to one's needs. Students, teachers and investigators are strongly urged to visit some great optical works like those of the Bausch & Lomb Optical Company in Rochester or the Spencer Lens Company in Buffalo and see with their own eyes the many and complicated processes that are necessary to produce a microscope. It is amazing that they can be made so well and so cheaply.

§ 57. **Stand of a research microscope.** — It should be rugged so that there will be the minimum give with the various manipulations. The foot should be large so that the microscope can be inclined to any angle without losing its balance.

Commencing below, the equipment should consist of a strong mirror fork with set-screw to hold the mirror in any desired position. The substage fitting should be centerable so that the substage condenser can be put in accurate axis alignment with the objective and ocular. It should be on a rack and pinion so that it may adjusted up and down; and if there is a special fine adjustment, it is of great advantage in the most critical work.

The stage should be of the circular, revolving type to enable the observer to put the object in any desired orientation for photography and for polarization.

There should be a mechanical stage which may be removed when a broad, free stage is demanded. It should have a wide excursion so that entire slides of serial sections can be studied.

The revolving nose-piece should be quadruple so that a battery of objectives may be used successively when necessary. It should be fixed to the movable focusing block, then the monocular and binocular bodies can be changed without disturbing the objectives or the focus of the microscope (fig. 37).

The monocular body should have a graduated draw-tube with a Society Screw at its lower end for very low objectives and for use with the apertometer (fig. 117).

A coarse and a fine adjustment are indispensable.

§ 58. Optical equipment of a research microscope. — There should, first of all, be a good mirror, concave on one side and plane on the other. If the microscope is to be used for fluorescence effects, the mirror should be of the first-surface type with the newly devised chromium-aluminum vapor film. Such a mirror is good also for all microscopic work. It reflects the ultra-violet and is much more convenient than the quartz prism reflector (§ 304).

Second, it should have an aplanatic, 1.40 N.A. condenser with a removable top element and central stops (fig. 282).

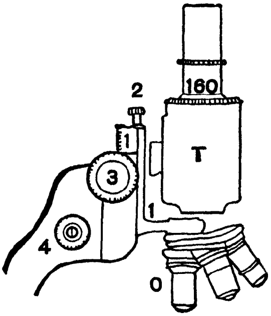


FIG. 37. DEVICE FOR CHANGING FROM BINOCULAR TO MONOCULAR BODIES WITHOUT DISTURBING THE REVOLVING NOSE-PIECE WITH ITS OBJECTIVES.

It was suggested by W. B. Carpenter in 1875 for the Stephenson binocular, and put in practice by Browning's modification in 1881, and Swift's in 1887.

1, 1 Bar attached to the movable focusing block for holding the nose-piece.

2 Screw to clamp the body in place.

3 Coarse adjustment.

4 Fine adjustment.

O Objectives in the nose-piece. Their position is not disturbed in changing bodies.

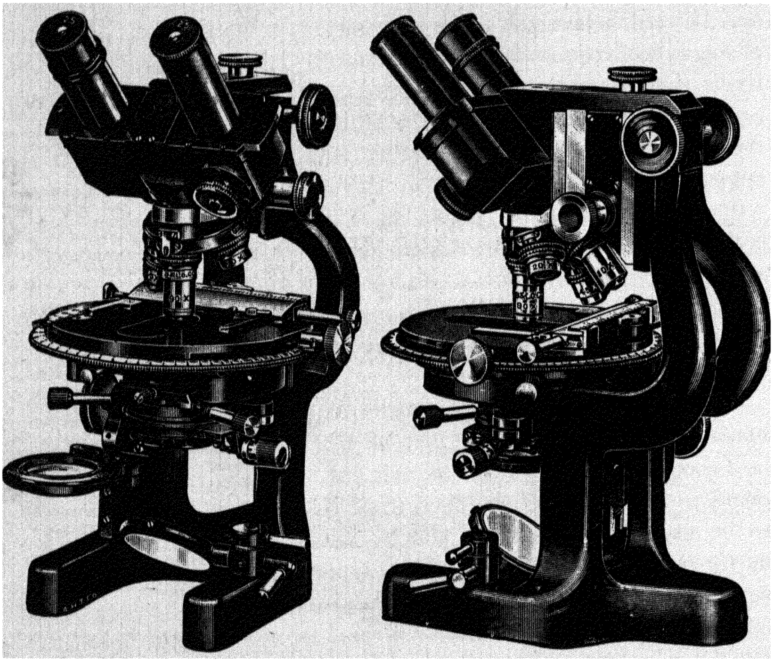
T Large body-tube.

160 Draw-tube at the 160 mm. tube-length mark.

Third, unless one has a dark-field microscope, there should be available one of the special dark-field condensers (figs. 74, 77, 84) to use in place of the aplanatic condenser.

Fourth, there should be some form of polarizing outfit unless one has a special polarizing microscope (fig. 92).

Fifth, one should be supplied with at least four dry objectives: a 40 or 32 mm., and a 16, 8, and 4 mm. There should be also an oil immersion, the 8 and 4 dry; and the oil immersion objectives should be supplied with iris diaphragms so that the aperture can be adjusted at will (Ch. III and XIV). As to the quality of these objectives, the achromatic modern objectives are excellent for general work and are relatively inexpensive. If one wishes to use objectives for the most



Front View *Side View*

FIG. 38. THE BAUSCH & LOMB MODEL DDE RESEARCH AND PHOTOGRAPHIC MICROSCOPE.

With this microscope the observer sits in front of the instrument, the light reaching the mirror from behind. A monocular body goes with it for photography. It is the first of the modern Mega-Microscopes.

(Line cuts through the courtesy of the Arthur H. Thomas Co.)

correct color value and for photomicrography, the fluorite and apochromatic objectives are superior but more expensive.

Sixth, the ordinary Huygenian oculars are good for the achromatic objectives. There should be one of 5x and one of 10x. If one must wear glasses, oculars with high eye-points, like the telaugics (§ 145) or the wide-field oculars of various manufacturers, are desirable. If apochromatic objectives are to be used, one must have a group of compensating eye-pieces; 5x, 10x and 15x are to be especially recommended. The 15x is particularly effective for dark-field work with all types of objectives.

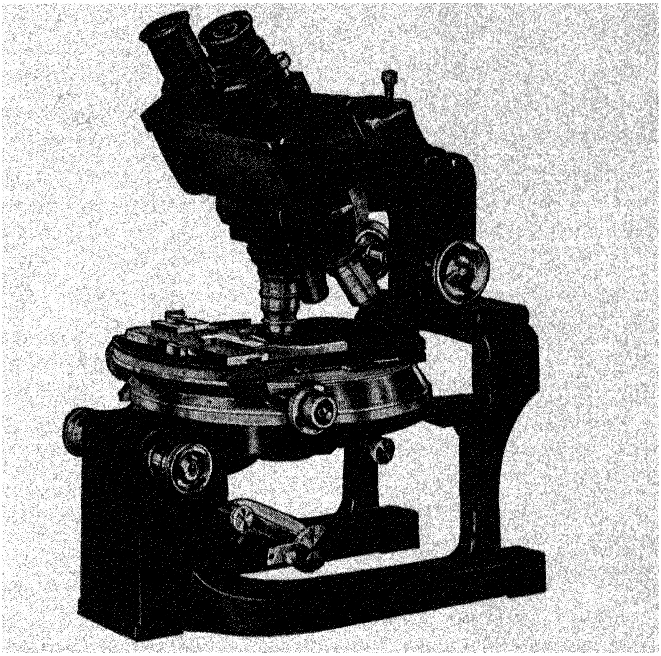


FIG. 39. THE NEW RESEARCH MICROSCOPE NO. 8 OF THE SPENCER LENS CO.

The observer sits in front of the stage, and the light source is behind the microscope. There is a single tube for photography.

(Courtesy of the Spencer Lens Co.)

Seventh, for micrometry one must possess some form of ocular micrometer. The one with drum and movable scale is most accurate and easily used (figs. 158–159; §§ 376–378). Of course, a stage micrometer is a necessity (§ 366).

§ **59. Mega-microscopes (figs. 38–39).** — During the last few years most elaborate research instruments have been devised by various manufacturers. These from their size and complexity may properly be called *mega-microscopes.* They have certain advantages in that there are present all the modern devices in one instrument. However, their size makes them unhandy to move about, and their very excellencies make them rather confusing; they are also very expensive. Finally, the possessor will probably find that they will not of themselves produce great discoveries; that, as stated above, depends on the brain of the observer.

Instead of one elaborate instrument to serve for all the needs of an investigator, it seems to the writer from much experience that it is better to have several instruments of rather simple fundamental design especially equipped for his various needs. Then each one can be kept in perfect adjustment and is available at a moment's notice. For example, there should be one fully equipped for bright-field work, one for the dark-field, one for polarization, and one for fluorescence effects. These four would not cost more than the single *mega-microscope* and could be purchased as needed in one's researches.

§ **60. General care of the microscope.** — The microscope should be handled carefully and kept clean. The oculars and objective should never be allowed to fall, for that might injure or displace their lenses. When not in use, the microscope should be covered or kept in a place as free as possible from dust. All parts of the microscope should be kept free from liquids, especially from acids, alkalies, alcohols, xylene, turpentine and chloroform.

§ **61. Care of the mechanical parts.** — To clean the sliding mechanical parts put a small quantity of some fine oil (olive oil or petrolatum and xylene, equal parts) on a piece of gauze or lens paper and rub the parts well; then with a clean, dry piece of cloth or lens paper wipe off most of the oil. If the sliding parts are kept clean in this way a special lubricator is rarely needed. In cleaning lacquered parts xylene alone answers well, but it should be quickly wiped off. Do not use alcohol, as it dissolves the lacquer.

§ **62. Care of the optical parts.** — These must be kept scrupulously clean in order that the best results may be obtained. Glass surfaces should not be touched by the fingers, for that almost invariably clouds them. Whenever an objective is left in position on the microscope or when several are attached to a revolving nose-piece, an ocular should be left in the upper end of the tube to prevent dust, lint, etc., from falling down upon the back lens.

As pointed out by Wright (p. 93), one of the surest ways to detect anything wrong with the objective is to examine the eyepoint with a magnifier. The field should be lighted well and the aperture of the objective filled about $\frac{2}{3}$ full of light. If there are any defects, as smears of balsam or liquids on the front lens, unsealing of the combinations, or dust on the upper face of the back lens, the defect can be seen in the eyepoint.

Another and very certain method of detecting imperfections is to rotate the different elements while looking into the microscope. If the defects are in the mirror, they will change in position when the mirror is moved, and so with all the other elements. Defects in the ocular are strikingly shown by rotating it.

§ **63. Lens paper.** — The so-called Japanese filter paper, which from its use with the microscope I have designated lens paper, has been used in the author's laboratory since 1884 for cleaning the lenses of oculars and objectives, and especially for removing the fluid used with immersion objectives. Whenever a piece has been used once it is then thrown away. It has proved more satisfactory than cloth or chamois because dust is not present and because of its bibulous character it is very efficient in removing liquid or semi-liquid substances. Some other workers with the microscope have found that absorbent cotton has the desired qualities for cleaning optical parts.

§ **64. Removal of dust, etc.** — (1) Dust may be removed with a camel's-hair brush, then the lens wiped with lens paper.

(2) Cloudiness may be removed from the glass surfaces by breathing on them, then wiping quickly with a soft cloth or the lens paper.

Cloudiness on the inner surfaces of the ocular lenses may be removed by unscrewing them and wiping as directed above. A high objective should never be taken apart by an inexperienced person.

If the cloudiness cannot be removed as directed above, moisten one corner of the cloth or paper with 95% alcohol, wipe the glass first with this, then with the dry cloth or the lens paper.

(3) Water may be removed with soft cloth or the lens paper.

(4) Glycerin may be removed with cloth or lens paper saturated with distilled water; remove the water as above.

(5) Blood or other albuminous material may be removed while fresh as in (4). If the material has dried on the glass, it may be removed more readily by adding a small quantity of ammonia to the water in which the cloth is moistened (water 100 cc., ammonia 1 cc.).

(6) In general, to remove any foreign substance from a glass surface a solvent of the foreign material must be used. For example, Canada balsam, damar, clarite, or cedar-wood oil is best removed from the front lens of an objective by wiping it with lens paper or a soft cloth moistened with xylene, toluene, or chloroform, and then wiping it dry with a fresh piece of lens paper or gauze.

(7) It frequently happens that the upper surface of the back combination of the objective becomes dusty. This dust may be removed in part by a brush but more satisfactorily by using a piece of the lens paper loosely twisted. When most of the dust is removed, some of the paper may be put over the end of a pine stick (like a match stick) and the glass surfaces carefully wiped. Sometimes it is necessary to moisten the wiper with water and then wipe dry.

§ **65. Care of the eyes.** — Keep both eyes open, using the eye-shade if necessary (fig. 40), and divide the labor between the two eyes, using

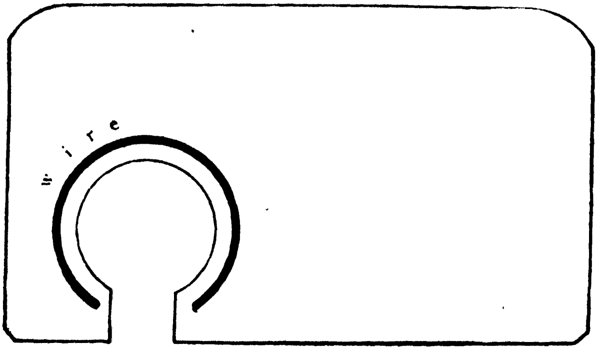


FIG. 40. EYE-SHADE FOR THE TOP OF THE MICROSCOPE TC ENABLE THE OBSERVER TO KEEP BOTH EYES OPEN.

(Devised by Lister; Quekett, pp. 170–171.)

one eye for a while and then the other. It frequently happens that one eye is much more perfect than the other, then, of course, the more perfect eye is used all the time (see Quekett, pp. 170-171).

The binocular microscope has certain advantages in that one uses both eyes all the time as in naked-eye observation. If a binocular is used, however, one must adjust it accurately so that each eye sees an equally sharp image (§ 163).

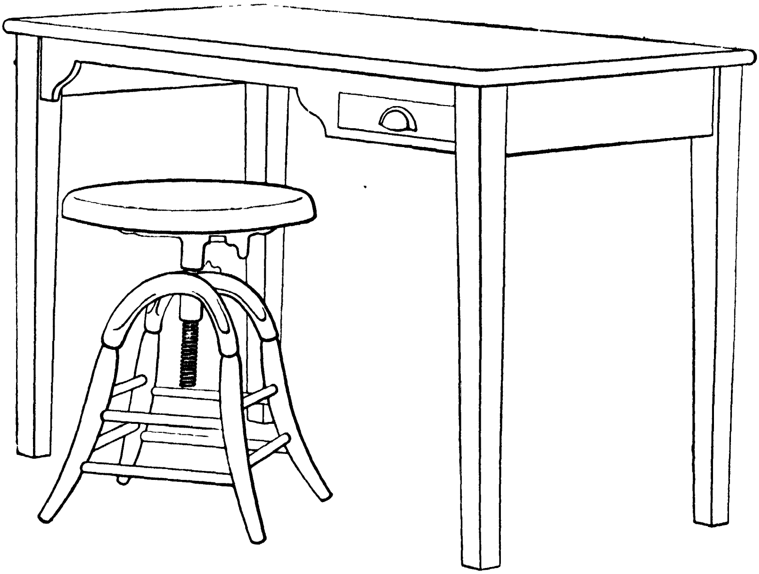


FIG. 41. LABORATORY TABLE AND ADJUSTABLE STOOL.

This table is 122 cm. long, 61 cm. wide, and 73 cm. high (2 × 4 feet on top, and 29 inches high).

The corners and edges are rounded and the top is stained with aniline black. The front of the rail is cut out, and the drawer is at the right so that it can be opened without moving the stool.

In the beginning it is not advisable to look into the microscope continuously for more than half an hour at a time. One never should work with the microscope after the eyes feel fatigued. After one becomes accustomed to microscopic observation he can work for several hours with the microscope without fatiguing the eyes. This is due to the fact that the eyes become inured to labor like the other organs of the body by judicious exercise. It is also due to the fact that but very slight accommodation is required of the eyes, the eyes remaining nearly in a condition of rest as for distant objects. The fatigue incident upon using the microscope at first is due partly at least to the constant effort on the part of the observer to remedy the defects of focusing the microscope by accommodation of the eyes. This should be avoided and the fine adjustment of the microscope used instead of the muscles of accommodation. With a microscope of the best quality, and suitable light — that is, light which is steady and not so bright as to dazzle the eyes nor so dim as to strain them in determining details — microscopic work should improve rather than injure the sight.

If artificial light is used, give it daylight qualities by placing a piece of daylight glass between the source of light and the microscope. This will give one a very soft light like that from a white cloud (§76).

§ 66. Position and character of the work-table. — The work-table should be very firm and large (61 × 122 cm. on top, and 73 cm. high; 24 × 48 × 29 in., figs. 41–41a), so that the necessary apparatus and material for work may not be too crowded. The table should also be of the right height to make work by it comfortable. An adjustable stool, something like a piano stool, is convenient; then one may vary the height corresponding to the necessities of special cases.

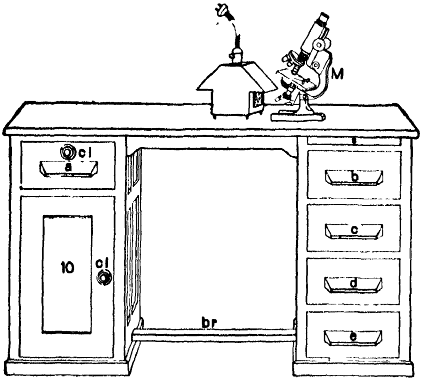


Fig. 41a. Microscopical Laboratory Desk with Microscope and Chalet Lamp.

(Desk designed by Dr. V. A. Moore; about one-twentieth natural size.)

The size of the top and the height are the same as for the laboratory table (fig. 41, § 66).

At the right there is a cabinet with combination lock (*10, cl*) for a microscope, and above a drawer with combination lock (*a, cl*).

At the right is a writing shelf (*s*) above the four drawers (*b, c, d, e*).

Near the bottom is a brace (*br*) which also serves as a foot rest.

M Compound microscope with the Chalet Microscope Lamp in front of it.

Other Reading

Nelson, E. M. — On the Origin of the Society Screw. J.R.M.S., 1910.

Riddell, J. L. — On the Binocular Microscope. Amer. Jour. Science, vol. 65, 1853, p. 68; Quarterly Jour. Micr. Science, vol. ii, 1854, pp. 18–24, 4 figs.

CHAPTER II

BRIGHT FIELD MICROSCOPES: LIGHTING, NATURAL AND ARTIFICIAL: EXPERIMENTS WITH MICROSCOPES.

§§ 67–169; FIGURES 42–64

§ **67. Bright-field lighting.** — With the great majority of microscopic work the objects are viewed on a light field, the general appearance being like dark or colored letters on a white sheet of paper. The light may be directed upon the surface, as in all ordinary vision with the naked eye, or the light may be made to shine through the support and the object from behind as in the glass signals for automobilists, or commercial signs on glass. When the microscope is used with a light field, it is called a bright-field microscope in contradistinction to a dark-field microscope where the object is bright and the field dark (§ 170).

§ **68. Lighting with daylight.** — Full sunlight is not used in ordinary work. North light is best and most uniform. When the sky is covered with white clouds, the light is most favorable. To avoid the shadows produced by the hands in manipulating the mirror, etc., it is better to face the light; but to protect the eyes and to shade the stage of the microscope some kind of screen should be used. The one shown in fig. 42 is cheap and efficient. If one dislikes to face the window or lamp it is better to sit so that the light will come from the left, as in reading.

It is of the greatest importance and advantage for one who is to use the microscope for serious work that he should comprehend and appreciate thoroughly the various methods of illu-

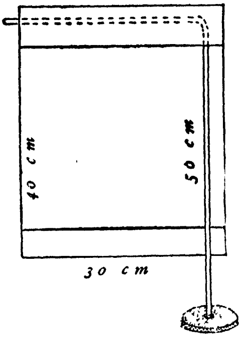


FIG. 42. SCREEN FOR SHADING THE MICROSCOPE AND THE OBSERVER.

It is composed of heavy paper hung over a bent wire, which in turn is anchored in a small tin dish filled with lead.

mination, and the special appearances due to different kinds of illumination.

§ **69. Reflected, incident, or direct light.** — By this is meant light reflected upon the object in some way and then irregularly reflected from the object to the microscope. By this kind of light objects are ordinarily seen by the unaided eye and the simple microscope (figs. 4–5). In histology, reflected light is but little used; but in the study of opaque objects, like whole insects, etc., it is used a great deal. For a simple microscope and low powers of the compound microscope, ordinary daylight that naturally falls upon the object, or is reflected or condensed upon it with a mirror, or a bull's eye condensing lens, is sufficient. For high powers, special apparatus is necessary. (See § 31).

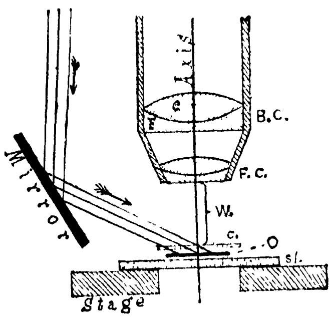


FIG. 43. LOW-POWER OBJECTIVE SHOWING WORKING DISTANCE AND REFLECTED LIGHT.

Axis The principal optic axis of the objective extended.

Sl The glass slip on which the object is mounted.

O Object.

c Cover-glass over the object.

W The working distance between the cover and the objective.

Mirror The mirror is represented as above the stage and reflecting parallel beams upon the object.

FC Front combination of the objective.

BC Back combination of the objective; it is composed of a plano-concave of flint (*F*) and a double convex lens of crown glass (*c*).

§ **70. Transmitted light.** — By this is meant light which passes through an object from the opposite side (figs. 20, 44). The details of a photographic negative are in many cases only seen or best seen by transmitted light, while the print made from it is best seen by reflected light (figs. 19, 43).

Almost all objects studied in animal and vegetable histology are lighted by transmitted light, and they are in some way rendered transparent or semi-transparent. The light traversing and serving to illuminate the object in working with a compound microscope is usually reflected from a plane or concave mirror, or from a mirror to a condenser, and thence transmitted to the object from below (fig. 18, 44).

§ **71. Axial or central light.** — By this is meant light reaching the object in such a way that it is symmetrically arranged around the optic axis of the microscope, then the object will be equally illuminated from all sides. If bundles of parallel rays are reflected upon the object from the mirror, they must be so disposed that the object will receive an equal quantity of light from all sides. If the bundles of light are made up of diverging or of converging cones, then the axes of the cones should be coincident with or parallel with and symmetrically arranged around the optic axis of the microscope.

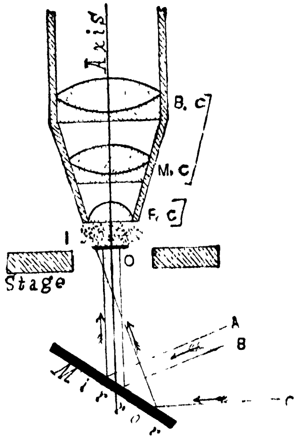


FIG. 44. HIGH-POWER IMMERSION OBJECTIVE WITH CENTRAL AND OBLIQUE TRANSMITTED LIGHT.

Axis The principal optic axis.

Mirror This reflects the light up through the object.

A B Central light.

C Oblique light.

Stage The microscope stage in section.

O The object.

I Immersion liquid between the objective and object.

F C The front lens of the objective.

M C The middle combination.

B C The back combination.

§ **72. Oblique light.** — By this is meant light which reaches the object with its axial beam oblique to the optic axis of the microscope. With oblique light the object cannot be illuminated equally from all sides, but largely from one side, and consequently the light is said to be unsymmetrical.

If no condenser is used, oblique light is obtained by turning the mirror so that parallel rays strike the object obliquely to the optic axis of the microscope (fig. 44c) or the axis of the converging or diverging beam from the concave mirror strikes the optic axis obliquely.

If a condenser is used, oblique illumination is produced by making the diaphragm opening eccentric, or most simply by putting the finger or other opaque body between the mirror and the condenser to cut off part of the light (figs. 62, 135). The result in all cases is that the object is lighted unsymmetrically.

§ **73. Use of a diaphragm.** — A diaphragm is an opaque disc with an opening, and is placed somewhere between the object and the source of light.

At the present time an iris diaphragm is almost universally employed. It, like the iris of the eye, can be expanded or contracted, and thus gives a large range of openings to meet different conditions.

The object of a diaphragm is to cut off adventitious light and to vary the aperture to suit the object and the objective.

§ **74. Size and position of the diaphragm with a mirror only.** — When no condenser is used in addition to the mirror, a diaphragm opening about the size of the front lens of the objective may be employed. Its position may be close to the object, in which case it admits the greatest aperture of light, and cuts off the most adventitious light. In this position it lights the smallest field, however.

If the diaphragm is far enough below the object, the field may all be lighted, but the aperture will be smaller than when it is close to the object, as one may see by removing the ocular and looking down the tube into the back lens of a 16 mm. (10x) or 8 mm. (20x) objective. On the other hand, while the aperture of the objective may be filled even with a small diaphragm opening close to the object, the field of view (§ 93, fig. 132) may be but partly lighted. In that case the opening must be increased until the entire field is illuminated. One must learn by practice how to get the best results.

ARTIFICIAL ILLUMINATION

§ **75. Artificial light.** — While daylight is preferred by many for all microscopic work, every one who must do much of that kind of work, realizes very keenly its defects. It continually varies in intensity and color from sunrise to sunset; and in most regions where work is done it is frequently cloudy or stormy and sufficient light is not obtainable. Then, too, it often happens that work should be continued into the evening when no daylight is available. Frequently, also, the worker must be in a room where suitable daylight cannot be secured, no matter how favorable the day may be. For all work it is advantageous to have a source that is uniform both in intensity and in color. This is especially necessary for photography. All forms of artificial light have been used at some time for microscopic work; and for a long time various means have been taken to make the artificial light as nearly like daylight as possible. This desire for artificial daylight is natural, for the eye was developed for daylight, and all its standards of color and shading have been worked out for that quality of light. In all of the ordinary forms of artificial light, the relative intensity toward the red end of the spectrum is much greater than with daylight, hence color values with artificial light are distorted, and with most people the excessive intensity of the red produces glare and a lack of contrast, which is trying to the eyes.

§ 76. **Artificial daylight.** — For the production of artificial daylight it is obvious from the curve (fig. 45) that there are two possible means: (1) The selection of two kinds of artificial light in which the lack in one is made good by the excess in another, and by mixing these in the right proportions the resulting light will have the same relative intensity in different parts of the spectrum as is found in sunlight. This is the "addative" method and has been quite successfully realized by combining a mercury arc light with its deficiency in the red, but its richness in intensity in the blue end of the spectrum, with a mazda incandescent lamp with its excessive red intensity. If these two lights are enclosed in a glass globe, and the right amount of each used, very good daylight is produced.

(2) As there is excessive intensity in the red part of the spectrum it is evident that if this excess can be absorbed by a light filter of some kind, then also the relative intensity of the light will be like that of natural daylight. This is the "subtractive" method, and is the method employed wherever a light filter or colored liquid, colored gelatin, colored glass, or a combination is used. From time immemorial various colored liquids like solutions of copper salts and colored glasses have been used to whiten the artificial light.

During the last few years, however, the problem has been solved, and now colored glass is made which gives to artificial light true daylight qualities. As each artificial light has its own special curve of intensity for the different parts of the spectrum, naturally a special light filter must be worked out for each light source. Up to the present, glass filters have been produced for the Welsbach gas

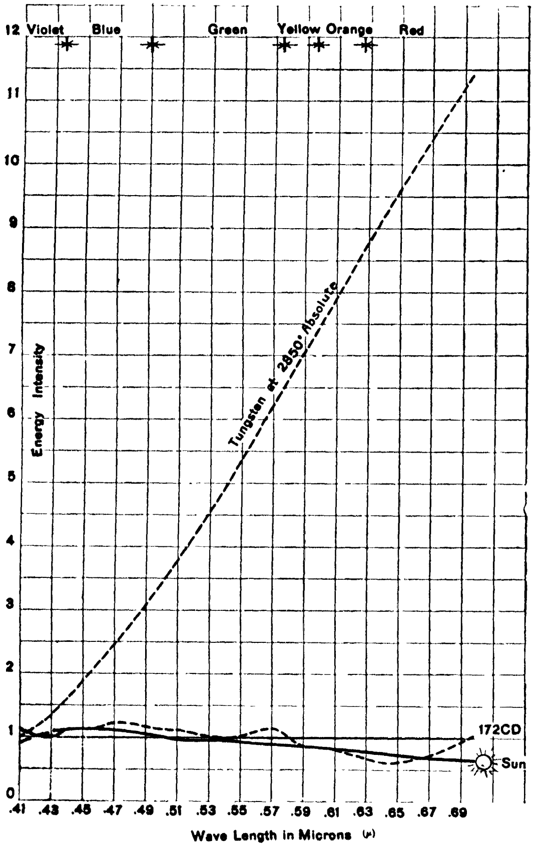


FIG. 45. CURVE OF ENERGY DISTRIBUTION IN SUNLIGHT; IN THE MAZDA C LAMP (TUNGSTEN AT 2800° ABSOLUTE); AND OF MAZDA LAMPLIGHT FILTERED THROUGH DAYLIGHT GLASS 172 CD. (H. P. GAGE)

light, and for the incandescent, nitrogen-filled tungsten (mazda) lamp. It may be said in passing that these glass filters whiten any artificial light, but that true daylight color values are given only under the precise conditions for which the glass was worked out. It is also gratifying to note that this successful solution of a long vexing problem came only when the rigid training in physics and chemistry and the facilities of a great manufacturing plant were brought together.

§ **77. Daylight-lamp or lantern.** — In the practical use of the daylight glass filter, it was found that the light should be enclosed in some kind of a lantern or lamp-house so that all the light delivered to the microscope might be of the daylight quality, and none of the unfiltered light scattered about the workroom.

After much experimenting, a lantern having the general form of a Swiss chalet was decided upon as it fulfilled all the requirements, and besides by its extending roof excluded all light from entering the eyes of the observer directly, one of the greatest causes of eye-fatigue. The old opticians and astronomers knew and stated well the conditions for the clearest vision, viz.; that no light should enter the eyes except that which came from the object being studied.

It was found also that the best effect was secured when the 100-watt lamp filament was opposite the middle of the window of daylight glass (ms fig. 46).

§ **78. The daylight-glass filter.** — Experience showed that the windows in the lamp-house or lantern should be about 82 mm. square in order to give sufficient area for lighting all the different powers from the lowest to the highest. It also served to supply light at the side of the microscope for drawing and note taking.

For the lower objectives, i.e., from the lowest up to the 4 mm. (40x), it is well to have one face of the glass filter ground with fine carborundum or emery flour to diffuse the light so that the image of the lamp filament will not show in the field. Formerly for these powers it was recommended that both faces be ground, but since at present all 100-watt, gas-filled lamp bulbs are inside frosted, it is necessary to grind only one face of the daylight filter to give the desired diffusion. For objectives of 3 mm. (60x) and less equivalent focus and higher powers, it is better to have one of the daylight glass windows smooth or polished like plate glass on both faces. If two students are to use the same lantern at the same time, then it is better to have both windows with one ground face. Even with the highest powers the ground glass window gives light enough if the lamp is brought close to the microscope. For powerful lamps to use with the dark-field microscope, for seeing the blood-circulation and for photography (figs. 78-82).

§ 78a. For a discussion of the requirements for the production of artificial daylight, and the means so far employed, and the uses of artificial daylight, see:

Herbert E. Ives. Artificial Daylight. Journal of the Franklin Institute, vol. 177, May, 1914, pp. 471-499. 19 figures.

Simon H. Gage. Artificial Daylight for the Microscope. Science, N. S., vol. 42, October, 1915, pp. 534-536. One curve.

M. Luckiesh. Artificial Daylight. Science, N. S., vol. 42, November, 1915, pp. 764-765.

Henry Phelps Gage. "Daylite Glass," a color screen for producing daylight artificially. The Sibley Journal of Engineering, Ithaca, N.Y., Vol. XXX, No. 8, May, 1916. 4 quarto pages, 6 figures.

Simon H. Gage and Benjamin F. Kingsbury. Some apparatus for the microscopical laboratory. Anatomical Record, Vol. X, No. 8, June, 1916, pp. 527-536. 7 figures showing the use of the daylight glass for microscopic work.

Anthony J. Brown. Some uses of artificial daylight in the psychological laboratory. American Journal of Psychology. July, 1916, Vol. XXVII, pp. 427-429.

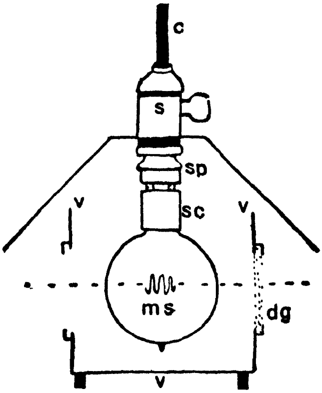


FIG. 46. CHALET MICROSCOPE LAMP IN SECTION.
(About one-fifth natural size.).

The metal part of the lantern or lamp-house is enameled white inside or preferably painted with aluminium powder in a suitable lacquer. The outside may be painted with any desired color of lacquer, or coated with black bakelite.

dg-dg Windows of daylight glass about 82 mm. square. One is ground on one surface with very fine emery or carborundum, to diffuse the light, and the other is left clear, or the glass may be polished on both faces.

ms mazda C lamp bulb of 100 watts. The filament of the lamp should be opposite the middle of the daylight window.

s The lamp socket with snap switch on the left, and the entering electric cable.

v v v Ventilating spaces at the top and at the bottom. The lamp-house has legs at each corner to elevate it and give free ventilation at the bottom. The roof is supported at the two ends and has ventilating spaces over the two walls containing the daylight filters, (figs. 53, 83, 198–199).

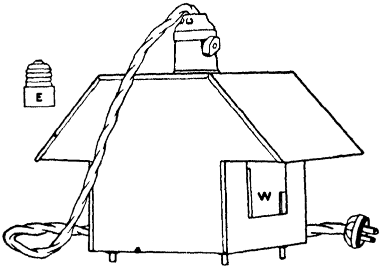


FIG. 47. NEW CHALET MICROSCOPE LAMP

The lamp-house is 1½ cm. lower than the one shown in fig. 46. This change was made to bring the shorter, new form of 100-watt mazda lamps at the right level with the window (W). E Plug to screw into the lamp socket of the supply wire.

EXPERIMENTS WITH SIMPLE AND WITH COMPOUND MICROCOPES

§ **79. Focusing a microscope.** — Focusing is mutually arranging an object and the microscope so that a clear image may be seen.

With a simple microscope either the object or the microscope or both may be moved in order to see the image clearly, but with the compound microscope the object more conveniently remains stationary on the stage, and the tube or body of the microscope is raised or lowered (fig. 26).

In general, the higher the power of the whole microscope, whether simple or compound, the nearer together must the object and the magnifier be brought.

§ **80. Focusing a simple microscope.** Use a reading glass, or any form of simple microscope such as the tripod magnifier (figs. 15, 16). Hold the magnifier over a printed page and look through the magnifier. The letters and words will appear as they do with the naked eye, but larger (fig. 4).

In order to get the sharpest image it will be necessary to raise and lower the magnifier until the best position is found. This mutual arrangement of magnifier and object is called focusing, or getting into focus.

§ **81. Size of the field.** — With any given magnifier, the size of the field, that is, the diameter of the area which can be seen at one time, can be determined by using the ten-centimeter rule as object and noting how many centimeters or millimeters can be seen at one time without moving the magnifier or the measure sidewise. It will also be found by trial that the greatest field can be seen when the eye is at the level of the eyepoint as with the compound microscope (§ 99).

LIGHTING WITH THE SIMPLE MICROSCOPE

§ **82. Opaque objects.** — For these the light strikes the surface and is reflected, mostly in an irregular manner so that the object can be seen almost equally well illuminated from any angle. Ordinarily the daylight falling upon the object will sufficiently illuminate it, also the light of a lamp.

Place a printed page in bright daylight or near a lamp where the light can shine upon it and then look at it with the simple microscope held in the hand, on the legs of the tripod (figs. 4, 15–17) or held by a special stand. By varying the distance between the microscope and the object one can soon find the best focus, and by changing the position of the object, the best position for the light available.

Of course if one wishes to discriminate colors precisely, daylight, natural or artificial, must be available.

§ **83. Transparent or semi-transparent objects.** — For these the light should pass through the object. Use a lantern slide or printing on very thin paper and hold it up toward the window or some artificial light with one hand, and with the other hold the magnifier. Look into the magnifier and move it toward and away from the object till a clear image is seen. Here the light passes through the object into the microscope and then to the eye instead of being reflected from the surface as in looking at the page of a book.

Experiments with the Compound Microscope

§ **84. Putting an objective in position and removing it.** — Elevate the tube of the microscope by means of the coarse adjustment (fig. 26) so that there may be plenty of room between its front or lower end and the stage. Grasp the objective lightly near its lower end with two fingers of the left hand, and hold it against the nut at the lower end of the tube or the revolving nose-piece (figs. 48–50). With two fingers of the right hand take hold of the milled ring near the back or upper end of the objective and screw it into the tube of the microscope or nose-piece. Reverse this operation for removing the objective. By following this method the danger of dropping the objective will be avoided.

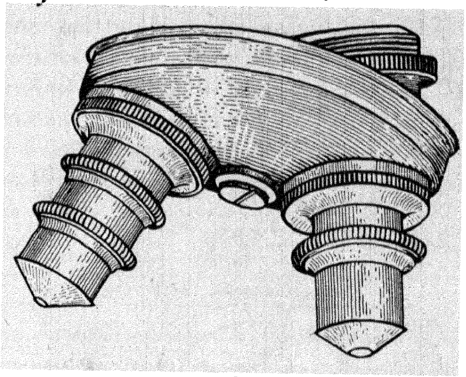


Fig. 48. Double Nose-piece with the Objectives in Place.

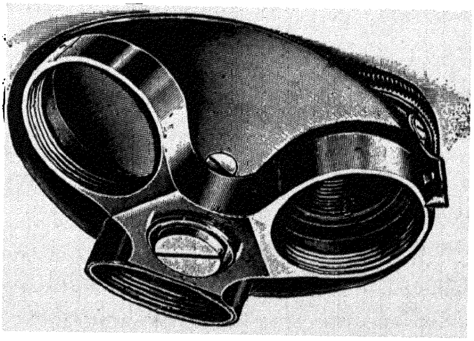


Fig. 49. Triple Nose-piece without Objectives.

Devices for quickly changing objectives of different powers date back nearly 150 years. (See Carpenter-Dallinger, 8th ed., 1901, pp. 290–295.)

§ **85. Putting an ocular in position and removing it.** — Elevate the body of the microscope with the coarse adjustment so that the objective will be 2 cm. or more from the object, grasp the ocular by the milled ring next the eye-lens (fig. 26) and the coarse adjustment or the tube of the microscope and gently force the ocular into position. In removing the ocular, reverse the operation. If the above precautions are not taken, and the oculars fit snugly, there is danger in inserting them of forcing the tube of the microscope downward and the objective upon the object.

§ **86. Putting an object under the microscope.** — This is so placing an object under the simple microscope, or on the stage of the compound microscope, that it will be in the field of view when the microscope is in focus (§§ 93, 79, fig. 40).

With low powers, it is not difficult to get an object under the microscope. The difficulty increases, however, with the power of the microscope and the smallness of the object. It is usually necessary to move the object in various directions while looking into the microscope, in order to get it into the field. Time is usually saved by getting the object in the center of the field with a low objective before putting the high objective in position. This is greatly facilitated by using a nose-piece, or revolver (figs. 48–50).

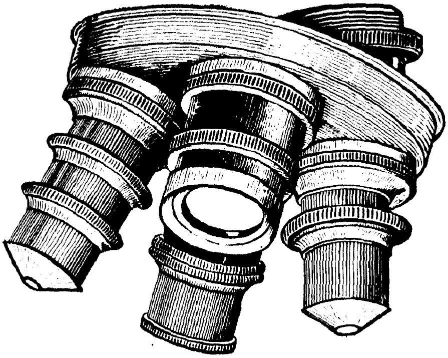


FIG. 50. QUADRUPLE NOSE-PIECE WITH THE FOUR OBJECTIVES IN PLACE.

FOCUSING EXPERIMENTS

§ **87. Focusing low objectives.** — Place a mounted fly's wing under the microscope; put the 16 mm. (10x) objective and the 5x or 6x ocular in position. Select the proper opening in the diaphragm and light the object well with transmitted light (§ 70).

Hold the head about the level of the stage, look toward the window, and between the object and the front of the objective; with the coarse adjustment lower the tube until the objective is within about half a centimeter of the object. Then look into the microscope and slowly elevate the tube with the coarse adjustment. The image will appear dimly at first, but will become very distinct by raising the tube still higher. If the tube is raised too high, the image will become indistinct, and finally disappear. It will again appear if the tube is lowered the proper distance.

When the microscope is well focused, try both the concave and the plane mirrors in various positions and note the effect.

Pull out the draw-tube 4 to 6 cm., thus lengthening the body of the microscope; it will be found necessary to lower the tube of the microscope somewhat. (For reason, see fig. 151.)

§ **88. Pushing in the draw-tube.** — To push in the draw-tube, grasp the large milled ring of the ocular with one hand, and the milled head of the coarse adjustment with the other, and gradually push the draw-tube into the tube. If this were done without these precautions the objective might be forced against the object and the ocular thrown out by the compressed air.

§ **89. Focusing with high objectives.** — Employ the same object as before, elevate the tube of the microscope and, if no revolving nose-piece is present, remove the 16 mm. (10x) objective as indicated. Put a 4 mm. (40x) or higher objective in place, and use 5x or 6x ocular.

Light well, and employ the proper opening in the diaphragm, etc. (§ 74). Look between the front of the objective and the object as before (§ 87), and lower the tube with the coarse adjustment till the objective almost touches the cover-glass over the object. Look into the microscope, and with the coarse adjustment, raise the tube very slowly until the image begins to appear, then turn the milled head of the fine adjustment (fig. 26), first one way and then the other, until the image is sharply defined.

In practice it is found of great advantage to move the preparation slightly while focusing. This enables one to determine the approach to the focal point either from the shadow or the color, if the object is colored. With high powers and scattered objects there might be no object in the small field (§ 93, fig. 51 for size of field). By moving the preparation an object will be moved across the field and its shadow gives one the hint that the objective is approaching the focal point. (See also § 81.) If one lowers the tube only when looking at the end of the objective as directed above, there will be no danger of bringing the objective in contact with the object, as may be done if one looks into the microscope and focuses down.

When the instrument is well focused, move the object around in order to bring different parts into the field. It may be necessary to refocus with the fine adjustment every time a different part is brought into the field. In practical work one hand is kept on the fine adjustment constantly, and the focus is continually varied.

§ 90. **Focusing with scattered or transparent objects.** — If the objects in a preparation are few or much scattered, or if they are unusually transparent it is sometimes difficult to find and focus them. To overcome the difficulty one can use a low power and get a specimen in the middle of the field. It is also advantageous in making such preparations if fresh to make a delicate cross (X) in the middle of the slide with India ink, or preferably a red glass pencil. It is then easy to focus the highest powers at the right level, when the scattered objects can be found by moving the slide. If a difficult preparation is permanently mounted, a delicate cross on the middle of the cover glass will aid one in getting the objects in focus.

The above suggestions will greatly assist with glass micrometers, fresh liquids like milk, blood, unstained bacteria, etc.

§ 91. **Parfocal oculars and focusing.** — On changing the oculars from a higher to a lower or the reverse, it is necessary to refocus the microscope. Formerly the change in focus was very marked in changing from one power of ocular to another, but since Mr. Pennock introduced parfocal oculars (1881) and their almost universal adoption since, very little change in focus is necessary in passing from power to power of ocular.

According to E. M. Nelson, such oculars were suggested by Varley and constructed by Powell as early as 1839 (Jour. Roy. Micr. Soc., 1908, p. 149).

§ 92. **.Parfocal objectives.** — These are groups of objectives, of different power, so mounted that when screwed into the revolving nose-piece of the microscope very little change in focusing is necessary in passing from objective to objective. This arrangement of objectives was a natural outgrowth from the parfocalization of the oculars, the ocular remaining the same (§ 91).

In case the objectives are not nearly enough parfocal so that the object is visible in turning from one objective to another, the defect can be corrected easily by getting one of the objectives in exact focus and then turning the others successively into place. If one notes whether it is necessary to focus up, then it will be known that the objective projects too far down toward the object; if, on the other hand, one must focus down, then the objective is too high up. To correct this lack of parfocalization use the objective which projects farthest toward the object as standard. Focus it sharply and then turn another in position. Unscrew this slowly until the image is also sharp. Now wind a thread or string around the lower end of the objective screw and then turn it in place and slowly screw it into the revolving nose-piece until it is in focus. Proceed with all until the entire number are in focus at the same level. With parfocal oculars and parfocal objectives much time and annoyance are saved, for one can see the specimen in turning from power to power, and it is necessary to make only a small focusing adjustment to get the best image. Microscope manufacturers prepare thin washers that can be put on top of the objectives for parfocalizing them. The washers are better than the string, but the string will answer if the washers are not at hand. While it is relatively simple to parfocalize different oculars, a group of objectives on a revolving nose-piece can

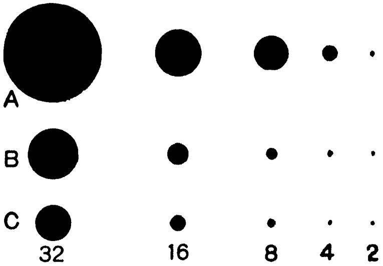


FIG. 51. FIELD WITH AND WITHOUT OCULARS AS SHOWN BY THE PROJECTION MICROSCOPE.

A The field of the 2 (90x), 4 (40x), 8 (20x), 16 (10x) and 32 (4x) mm. objectives without an ocular.

B Field of the same objectives with a 5x ocular.

C Field of the same objectives with a 10x ocular.

32 (4x), 16 (10x), 8 (20x), 4 (40x), 2 (90x). Equivalent focus of the different objectives whose fields are shown.

be accurately parfocalized for only one ocular. The 10x ocular is generally selected. Fortunately when parfocal for the 10x ocular, the objectives will not be far from parfocal for the 15x and the 6x, the other oculars most used.

§ **93. Field or field of view of a microscope.** — This is the area visible through a microscope when it is in focus. When the field is properly lighted and there is no object under the microscope, it appears as a disc of light. When examining an object, it appears within the light circle, and by moving the object, if it is of sufficient size, different parts are brought successively into the field of view.

In general, the greater the magnification of the entire microscope, whether the magnification is produced mainly by the objective, by the ocular, by increasing the tube-length, or by a combination of all three (§ 368), the smaller is the field.

The size of the field is also dependent, in part, without regard to magnification, upon the size of the opening in the ocular diaphragm. Some oculars, as the orthoscopic and periscopic, are so constructed as to eliminate the ocular diaphragm, and in consequence, although this is not the sole cause, the field is considerably increased.

§ **94. Method of determining the size of the field, and table with different objectives and oculars.** — Use a stage micrometer (fig. 148) as object, and read off the number of spaces required to measure the diameter of the light disc as seen in the microscope. Use first a low objective 16 mm. (10x) and a low ocular (5x or 6x), then use a higher ocular (10x or 15x). Do the same with the 4 (40x) or 8 (20x) mm. objective and the two oculars. Make a table giving the diameter of the field in each case and compare with the accompanying table. The tube-length (fig. 26) should be 160 mm. when making the measurements. To see the effect of lengthening the tube, pull it out as far as possible and note the effect on the size of the field. (The longer the tube, the smaller the field).

FUNCTION OF AN OBJECTIVE

§ **95.** Put a 50-mm. (3.2x) objective on the microscope, or screw off the front combination of a 16 mm. (10x) and put the back combination on the microscope for a low objective. For object, use some letters or numerals printed on thin paper and mounted in Canada balsam (§ 330). Place on the stage so that they are erect to the naked eye. Light as brilliantly as possible with transmitted light.

Differences in the magnification and the diameter of the field with the same objective but with different oculars having the same designation.

Achromatic objective of 16 mm. equivalent focus (e.f.) 10x	5x Huygenian (negative) oculars		10x Huygenian (negative) oculars		10x Positive oculars		15x Positive oculars	
	Field	Mag.	Field	Mag.	Field	Mag.	Field	Mag.
16 mm. 10x	1.95 mm.	61.2x	1.48 mm.	97.5x	1.95 mm.	96x	0.95	168x
16 mm. 10x	2.1 mm.	54x	1.21 mm.	105x	1.67 mm.	100x	1.17	147x
16 mm. 10x	2.3 mm.	51.2x	1.40 mm.	105x	1.70 mm.	96x	1.20	131x
16 mm. 10x	1.98 mm.	60x	1.53 mm.	105x	1.67 mm.	109x	1.17	139x
16 mm. 10x	1.85 mm.	60x	1.45 mm.	115x	1.52 mm.	109.2x	0.98	168x
16 mm. 10x			1.64 mm.	96x	1.10 mm.	113.2x	1.45	160x
16 mm. 10x			1.27 mm	102x				

Differences in the diameter of the field and the magnification of different objectives but with the same ocular.

Huygenian (negative) ocular, 10x	16 mm. (e.f.) 10x objectives		8 mm (e.f.) 20x objectives		4 mm. (e.f.) 40x objectives		3 mm. (e f.) 60x objectives	
	Field	Mag.	Field	Mag.	Field	Mag.	Field	Mag.
10x	1.60 mm.	96x	0.80 mm.	196x	0.37 mm.	425x	0.27 mm.	580x
10x	1.67 mm.	91.6x	0.85 mm.	185x	0.37 mm.	431x	0.26 mm.	586x
10x	1.60 mm.	95.2x	0.76 mm.	203x	0.39 mm.	420x	0.27 mm.	576x
10x	1.65 mm.	94x	0.80 mm.	195x	0.42 mm.	368x		
10x	1.64 mm.	96x	0 94 mm.	168x				
10x	1.53 mm.	102.8x	0.74 mm.	212x				

It is evident from the above tables that the magnification of the microscope obtained by multiplying the designated magnification of the objective and of the ocular would be only roughly correct. For the exact magnification, that for every combination must be individually determined.

In place of an ocular put a screen of ground-glass, or a piece of lens paper, over the upper end of the tube of the microscope.

Lower the tube of the microscope by means of the coarse adjustment until the objective is within 2 to 3 cm. of the object on the stage. Look at the screen on the top of the tube, holding the head about as far from it as for ordinary reading, and slowly elevate the tube by means of the coarse adjustment until the image of the letters appears on the screen.

The image can be seen more clearly if the object is in a strong light and the screen in a moderate light, i.e., if the top of the microscope is shaded.

The letters will appear as if printed on the ground-glass or paper, but will be inverted.

If the objective is not raised sufficiently, and the head is held too near the microscope, the objective will act as a simple microscope. If the letters are erect, and appear to be down in the microscope and not on the screen, hold the head farther from it, shade the screen, and raise the tube of the microscope until the letters do appear on the ground-glass.

95a. Ground-glass may be very easily prepared by placing some fine emery or carborundum between two pieces of glass, wetting it with water, and then rubbing the glasses together for a few minutes. If the glass becomes too opaque, it may be rendered more translucent by rubbing some oil upon it.

§ 96. **Aërial image.** — After seeing the real image on the ground-glass or paper, use the lens paper over about half of the opening of the tube of the microscope. Hold the eye about 250 mm. from the microscope as before and shade the top of the tube by holding the hand between it and the light, or in some other way. The real image can be seen as if in part on the paper and in part in the air. Move the paper so that the image of a letter will be half on the paper and half in the air. Another striking experiment is to have a small hole in the paper placed over the center of the tube opening; then if a printed word extends entirely across the diameter of the tube, its central part may be seen in the air, the lateral parts on the paper. The advantage of the paper over part of the opening is to enable one to accommodate the eyes for the right distance. If the paper is absent, the eyes adjust themselves for the light circle at the back of the objective, and the aërial image appears low in the tube. Furthermore, it is more difficult to see the aërial image in space than to see the image on the ground-glass or paper, for the eye must be held in the right position to receive the rays projected from the real image, while the granular surface of the glass and the delicate fibers of the paper reflect the rays irregularly, so that the image may be seen at almost any angle, as if the letters were actually printed on the paper or glass.

The function of an objective, as seen from these experiments, is to form an enlarged, inverted, real image of an object, this image being formed on the opposite side of the objective from the object (figs. 13, 18).

FUNCTION OF AN OCULAR

§ **97.** Using the same objective as for § 95, get as clear an image of the letters as possible on the lens paper or ground-glass screen. Look at the image with a simple microscope (fig. 15), as if the image were an object.

Observe that the image seen through the simple microscope is merely an enlargement of the one on the screen, and that the letters remain inverted. Remove the screen and observe the aërial image with the tripod magnifier.

Put 5x ocular, i.e., an ocular of low magnification in position (§ 85). Hold the eye about 10 to 20 mm. from the eyelens and look into the microscope. The letters will appear as when the simple microscope was used (see above); the image will become more distinct by slightly raising the tube of the microscope with the coarse adjustment.

The function of the ocular, as seen from the above, is that of a simple microscope, viz., it magnifies the real image formed by the objective as if that image were an object. Compare the image formed by the ocular (figs. 2, 18) and that formed by a simple microscope (figs. 1, 6).

It should be borne in mind, however, that the rays from an object as usually examined with a simple microscope extend from the object in all directions, and no matter at what angle the simple microscope is held, provided it is sufficiently near and points toward the object, an image may be seen. The rays from a real image, however, are continued in certain definite lines and not in all directions; hence, in order to see this aërial image with an ocular or simple microscope, or in order to see the aërial image with the unaided eye, the simple microscope, ocular, or eye must be in the path of the rays (figs. 1, 2).

§ **98.** The field lens of a Huygenian and other negative oculars makes the real image smaller and consequently increases the size of the field; it also makes the image brighter by contracting the area of the real image (figs. 24, 25). Demonstrate this by screwing off the field lens and using the eyelens alone as an ocular, refocusing if necessary. Note that the image is bordered by a colored haze.

When looking into the ocular with the field lens removed, the eye should not be held so close to the ocular, as the eyepoint (fig. 24) is considerably farther away than when the field lens is in place.

§ **99. Eyepoint.** — This is in the plane above the ocular where the emerging rays cross (figs. 22–25). If the eye is placed at this point it will receive the greatest number of rays from the microscope and thus see the largest field. If the eye is too far from or too near the ocular, part of the rays cannot enter the pupil of the eye and the microscopic image is restricted.

Demonstrate the eyepoint by using a 16 mm. (10x) objective and a 5x ocular. Light brightly and then focus the microscope on some transparent specimen. Open the diaphragm widely so that the entire aperture of the objective is filled with light (fig. 61). Shade the ocular with the hand or a screen and hold above the eyelens a piece of ground-glass or of the lens paper. By raising and lowering the glass or paper one will find the level where the sharpest and brightest light circle is located. The height varies with different oculars.

The eyepoint is also known as the *pupil of the lens;* exit pupil; Ramsden disc or circle; or Lagrange disc.

One can find the eyepoint of a simple microscope by placing it on the top of the tube of the compound microscope after removing the ocular. Then a piece of ground-glass or of lens-paper is held over the simple microscope and moved up and down until the brightest point is found. This is the eyepoint, and if the eye is at that level in looking into the simple microscope or magnifier, the largest field can be seen.

§ 100. Erect and inverted images with the microscope. — By glancing at fig. 1, 6, it will be seen that with the simple microscope the retinal image is inverted; that is, the arrow is turned end for end. In like manner the retinal image of any object seen with the naked eye is also inverted (fig. 5).

On the other hand, with the compound microscope, the retinal image is erect (figs. 2, 18); that is, the arrow points in the same direction as the object. This happens because the eye does not see the object directly, but the real image formed by the objective, and this is inverted. From the crossing of the rays on entering the eye, this inverted real image is reinverted, and thus gives an erect image on the retina. Now as objects or their images do not seem to be on the retinal screen, but out in space in the direction of the light rays entering the eye, it is very evident that if the light rays are traced from the retinal image to the object or to a virtual image, this will appear to be erect when the image on the retina is inverted as with the simple microscope, and will appear inverted when the retinal image is erect as with the compound microscope, because of the crossing of the rays in passing the pupil of the eye (figs. 1, 2, 6, 18) on their way to the retinal image, or on their way from the retinal image to the apparent position of the object or the virtual image.

WORKING DISTANCE

§ 101. Working distance. — By this is meant the space between the simple microscope and the object, or between the front lens of the compound microscope and the object, when the microscope is in focus. This working distance is always considerably less than the equivalent focal length of the objective. For example, the front-lens of a 4 mm. (40x) objective would not be 4 millimeters from the object when the microscope is in focus, but considerably less than that distance, viz., less than half a millimeter. If now a cover-glass of half a millimeter or more in thickness were used it would be impossible to get the 4 mm. (40x) objective near enough the object to get it in focus.

§ 102. Free working distance. — (1) Where no cover-glass is used, this is the distance between the front of the magnifier or the front lens mount of the objective and the object (fig. 52 *A*).

(2) If a cover-glass is used, it is the distance between the upper surface of the cover-glass and the magnifier or objective when the microscope is in focus (figs. 52 *B*, 43). Strictly speaking, it is the distance between the objective front and the upper surface of a cover-glass of the exact thickness for which the objective is corrected.

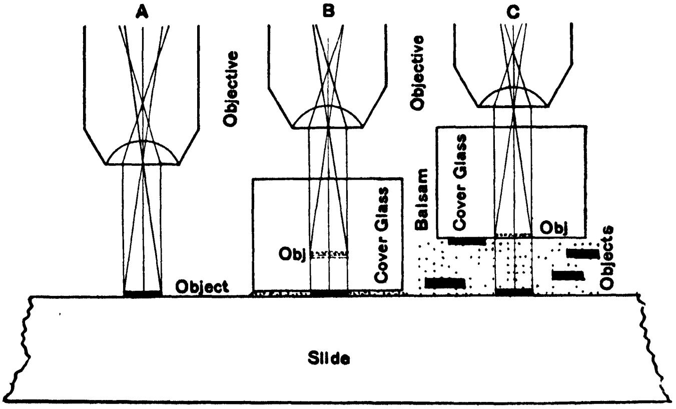


FIG. 52. WORKING DISTANCE AND THE COVER-GLASS.

Slide The glass slide upon which the object is mounted.
A Working distance with an uncovered object.
B Working distance when a cover-glass is used and the object is in contact with the cover-glass. The object represented by the solid black oblong appears to be elevated one-third the thickness of the cover to the level *Obj.*, where it is represented by dots.
The objective is elevated corresponding to the apparent elevation of the object.
C Working distance when a cover-glass is used and the objects are distributed in a stratum of Canada balsam.
It is evident from this figure why the focus must be different for objects at different depths in the balsam.

As the working distance of an objective is practically always less than its equivalent focus, one must take care to use cover-glasses thin enough so that any suitable objective can be used for studying the specimen. Furthermore, as microscopic specimens have considerable thickness, the cover-glass should be thin enough so that the objective can be lowered sufficiently to enable one to bring the lower strata of the specimen in focus without bringing the objective front in contact with the upper surface of the cover-glass (fig. 52 *C*).

§ **103. Determination of working distance, no cover.** — Some manufacturers state this in the description of their objectives. The information serves as a guide, for if a cover thicker than this working distance is used, the objective cannot be put in focus. Occasionally students and even experienced workers put unlabeled slides under the microscope wrong side up. With low powers the specimen can be focused through the thickness of the slide, but the high powers cannot, because the slide thickness is greater than the working distance. The working distance is always less than the equivalent focus of the objective because the center of the lens combination is some distance above the lower face of the front lens.

To determine the distance with low powers make a wooden wedge 10 cm. long which shall be exceedingly thin at one end and about 20 mm. thick at the other. Place a slide on the stage and some dust or an ink or pencil mark on the slide. Do not use a cover-glass. Use a 16 mm. (10x) objective and focus the dust or mark carefully, and when the objective is in focus, push the wedge between the objective and slide until it touches the objective. Mark the place of contact with a pencil and then measure the thickness of the wedge with a rule opposite the point of contact. This thickness will represent very closely the working distance. For measuring the thickness of the wedge at the point of contact for the high objective, use a steel scale ruled in $\frac{1}{5}$ mm. and the tripod magnifier to see the divisions. Or one may use a cover-glass measurer (§ 518) for determining the thickness of the wedge.

For the higher powers, if one has a microscope in which the fine adjustment is graduated, the working distance may be readily determined as follows:

Use the marked slide as above. Get the dust or mark in focus, then lower the tube of the microscope until the front of the objective just touches the slide. Note the position of the micrometer screw and slowly focus up with the fine adjustment until the dust or mark is again in focus. By noting the total and partial revolutions of the graduated fine adjustment the working distance will be known. For example, suppose it required 5.5 revolutions of the micrometer screw to raise the objective from the surface of the slide where the object is located to a point where the microscope is in focus, and the micrometer screw raises the objective 0.1 mm. for each complete revolution, then the total elevation will be 0.1 × 5.5 = 0.55 mm., that is, the working distance in this case is 0.55 millimeter.

§ **104. Free working distance in covered objects.** — Use a 4 mm. (40x) objective and the fly's wing or any covered object. Set the fine adjustment head at zero (0). Lower the objective carefully with the coarse adjustment until the objective just touches the cover-glass. Now focus up with the fine adjustment until the object is in sharp focus, noting the total and partial revolutions of the screw to accomplish this. The distance the objective was raised is the free space between the front of the objective and the cover-glass. Suppose it required 3.2 revolutions of the fine adjustment to focus the objective, then if each revolution represents 0.1 mm. the total elevation is 3.2 × 0.1 = 0.32 mm. for the free working distance in this case.

§ **105. Effect of the cover-glass on the working distance.** — It is obvious that if an object is covered with a layer of glass, the free space between the front of the objective and the object will be lessened, and if the layer of glass is considerably thicker than the working distance of the objective, then it will be impossible to get the object in focus. If the layer of glass is relatively thin, then it will be possible to focus the microscope on the object, but from the law of refraction it necessarily follows that the focus of the microscope with and without a cover-glass will not be the same.

Now from the refraction of the rays in passing from one medium to another of different refractive power, it follows that, when an object is in or below a stratum of glass or water or other highly refractive body, the object will appear as if raised (figs. 52 *B*, 64), the amount of the apparent elevation depending on the refractive index of the covering body, — the greater its refraction, the more the apparent elevation. The general physical law is that, the eye being in the air, the apparent depth of an object below the surface when viewed perpendicularly is the actual depth multiplied by the reciprocal of the index of refraction of the covering body. The index of refraction of the cover-glass is 1.52 or approximately 1.50, and its reciprocal is $\frac{1}{1.5} = \frac{2}{3}$. That is, the apparent depth is only $\frac{2}{3}$ its actual depth, or in other words the object seems to be elevated $\frac{1}{3}$ of the actual depth.

Now if the object is apparently higher up, the microscope must be raised an amount equal to the apparent elevation of the object. This is illustrated in figs. 52 *B–C*. From this it follows that the free working distance of the objective on a covered object is not lessened the full thickness of the cover-glass, but only $\frac{2}{3}$ of that thickness.

§ 106. Demonstration that the working distance is lessened only $\frac{2}{3}$ the thickness of the cover-glass. — Use a clean, flat glass slide. Put an ink or pencil mark on the upper face for object. Employ a 16 mm. (10x) objective and a 10x ocular. Focus the microscope on the ink or pencil mark, then measure the free space between the slide and the end of the objective with the wooden wedge, as directed in § 103. This is the free working distance (§ 102) without a coverglass.

Cut a glass slide up into two or three pieces for cover-glasses. Measure the thickness of one of the pieces with the cover-glass measurer or in some other good way. Place this over the mark on the slide which was in focus. If now one looks into the microscope, the mark will not be in focus with the glass cover over it. Focus up carefully until the mark is again in focus. Measure the space between the top of the cover-glass and the objective as before. This will represent the free working distance with this cover-glass.

Subtract the free working distance with this cover-glass from that with no cover-glass and the difference will be the amount the free working distance has been lessened by the addition of the cover. This amount compared with the thickness of the cover-glass will give the ratio of lessening of working distance by the addition of the cover-glass.

In an actual case the results were as follows:

Free working distance without cover..................	4.62 mm.
“ “ “ with cover......................	3.54 mm.
Lessening of the working distance by the cover-glass.....	1.08 mm.
The actual thickness of the cover-glass was............	1.62 mm.

That is, the lessening of the free working distance was not so great as the thickness of the cover (1.62 mm.), but less; viz. 1.08 mm.; that is, in the proportion of $\frac{1.08}{1.62} = \frac{2}{3}$ of the actual thickness of the cover-glass.

§ **107. Determining the thickness of the cover-glass with mounted objects.** — From what has been learned about the free working distance with covered objects, it is possible to determine the thickness of the cover-glass over an object if the object is in contact with the cover. If it is below, as shown in fig. 52 *C*, and the mounting medium is Canada balsam with approximately the same refractive index as glass, then it is possible to determine how great is the combined thickness of the cover-glass and layer of Canada balsam over the object.

Demonstrate the method as follows: (1) Where the object is in contact with the lower surface of the cover-glass (fig. 52 *B*). Use a 4 mm. (40x) objective and a cover-glass $\frac{15}{100}$ mm. thick. Make a black ink mark on one side of the cover and a colored ink mark directly opposite on the other side of the cover, or use glass pencils of two colors. Set the graduations of the fine adjustment at zero (0). Place the marked cover on a glass slide, and put under the microscope. Focus with the coarse adjustment on the mark at the upper surface of the cover. Then focus down with the fine adjustment until the mark on the lower surface appears sharp. For verification, focus up until the upper mark is again sharp. The elevation will of course be the same as the lowering. If the total and partial revolutions of the fine adjustment screw are noted, they will show how much the objective was lowered to get the lower mark in focus. In the case here given it was lowered 1 revolution. Now as each revolution moves the objective up or down 0.1 mm., the objective was moved down 0.1 or $\frac{10}{100}$ of a millimeter. As this represents $\frac{2}{3}$ of the thickness of the cover from the effect of refraction, the whole thickness must be $0.10 \div \frac{2}{3} = 0.15$ mm. For a cover of unknown thickness with the object in contact with its under surface, put an ink mark on the upper surface of the cover and proceed exactly as above, focusing successively on the object and on the ink spot.

(2) Where the object is somewhere below the cover-glass (fig. 52*C*). In this case the thickness of the cover-glass cannot be determined, but one can determine very approximately the combined thickness of the cover-glass and the mounting medium over the object as follows: Put an ink or glass pencil mark on the upper surface of the cover-glass. Focus the mark with the coarse adjustment after setting the graduations of the fine adjustment at zero (0). Then focus down with the fine adjustment until the object is sharp. Note the number of revolutions and the partial revolution of the fine adjustment drum. As this amount represents only $\frac{2}{3}$ of the actual thickness of the glass and mounting medium over the object, divide the observed amount of movement by $\frac{2}{3}$ and the quotient will represent the total thickness over the object.

For example, in one case the microscope was focused on the ink mark at the top of the cover, and then it was necessary to focus down $1\frac{1}{2}$ revolutions of the fine adjustment screw to bring the object in focus. That is, it was necessary to focus down 0.15 mm. As this represents but $\frac{2}{3}$ of the actual thickness of the cover-glass over the object, the entire thickness must be $(0.15 \div \frac{2}{3})$ $\frac{2}{3}$ or 0.225 mm. But as the specimen was mounted in balsam which has nearly the refractive power of glass, it represents the combined thickness of cover-glass and balsam mounted object. Probably in this case the cover-glass was 0.15 mm. and the object 0.075 mm.

LIGHTING EXPERIMENTS WITH THE COMPOUND MICROSCOPE

§ 108. Daylight with a mirror. — As the following experiments are for mirror lighting only, remove the substage condenser if one is present (see § 114, for condenser). Place a mounted fly's wing under the microscope, put the 16 mm. (10x) or other low objective in position, also a 5x ocular. With the coarse adjustment lower the tube of the microscope to within about 1. cm. of the object. Use an opening in the diaphragm about as large as the front lens of the objective; then with the plane mirror try to reflect light up through the diaphragm upon the object. One can tell when the field (§ 93) is illuminated by looking at the object on the stage, but more satisfactorily by looking into the microscope. It sometimes requires considerable manipulation to light the field well. After using the plane side of the mirror turn the concave side into position and light the field with it. As the concave mirror condenses the light, the field will look brighter with it than with the plane mirror. It is especially desirable to remember that the excellence of lighting depends in part on the position of the diaphragm (§ 73). If the greatest illumination is to be obtained from the concave mirror, its position must be such that its focus will be at the level of the object. This distance can be determined very easily by finding the focal point of the mirror in full sunlight.

§ 109. Use of the plane and of the concave mirror. — The mirror should be freely movable, and have a plane and a concave face (fig. 18). The concave face is used when a large amount of light is needed, the plane face when a moderate amount is needed or when it is necessary to have parallel rays or to know the direction of the rays.

§ 110. Axial or central light (§ 71). — Place a preparation containing minute air bubbles under the microscope. The preparation may be easily made by beating a drop of mucilage on the slide and covering it. (See § 334.) Use a 4 mm. (40x) objective and a 5x ocular. Focus the microscope and select a small bubble, one whose image appears about 1 mm. in diameter, then arrange the plane mirror so that the light spot in the bubble appears exactly in the center. Without changing the position of the mirror in the least, replace the air bubble preparation by one of *Pleurosigma angulatum* or some other finely marked diatom. Study the appearance very carefully.

§ 111. Oblique light (§ 72). — Swing the mirror far to one side so that the rays reaching the object may be very oblique to the optic axis of the microscope. Study carefully the appearance of the diatom with the oblique light. Compare the appearance with that where central light is used. The effect of oblique light is not so striking with histological preparations as with diatoms.

It should be especially noted in §§ 110–111, that one cannot determine the exact direction of the rays by the position of the mirror.

This is especially true for axial light (§ 110). To be certain the light is axial some such test as that given in § 334 should be applied.

EXPERIMENTS WITH ARTIFICIAL LIGHT AND A MIRROR

§ **112. Lighting with a kerosene lamp.** — For this a lamp with a flat wick from 3 to 5 cm. wide is best. It should be turned up well, but not enough to smoke. The face of the flame should be turned toward the microscope for low powers. For moderate powers the flame should be made oblique, and for high powers the edge of the flame should be used. This is because the thicker source of light gives a greater brilliancy. Use the fly's wing or any well-stained preparation.

As the light is in diverging beams, it is best to use the concave mirror to partly overcome the divergence. One must learn by experience and trial how far off to have the lamp. A distance of 15 to 20 cm. is usually satisfactory. There should be an opaque screen between the lamp and the microscope to protect the eyes of the observer and to screen the stage of the microscope (fig. 42).

This lamp illumination is brilliant, but the color values are quite unlike those given by daylight.

§ **113. Lighting with artificial daylight.** — For the source of light use preferably a 100-watt nitrogen-filled mazda lamp enclosed in a kind of lantern (figs. 46, 53). Have the lamp filament at about the level of the center of the microscope mirror, and a frosted disc of daylight glass, before an aperture in the lantern.

For object, use a fly's wing or any good, well-stained specimen. It would be interesting to sit near a window, and to turn the mirror in such a way as to bring in daylight a part of the time. In this way one can get a good idea of the real similarity of the artificial and of the natural daylight. If one also had an electric lamp without any light filter one could pass in order from real daylight, through the artificial daylight and then on to the unmodified artificial light. Without seeing these in comparison, one is hardly able to appreciate the likeness between the natural and artificial daylight and the great unlikeness of unfiltered electric light and artificial daylight.

§ **114. Condensers.** — These are single lenses or lens systems to aid in illuminating objects by either direct or transmitted light (§§ 69–70). For direct or reflected light, such as is required for opaque objects, condensers are usually simple, and are called "bull's-eyes." They are mounted on a stand for holding them at different heights and in any desired position (fig. 127).

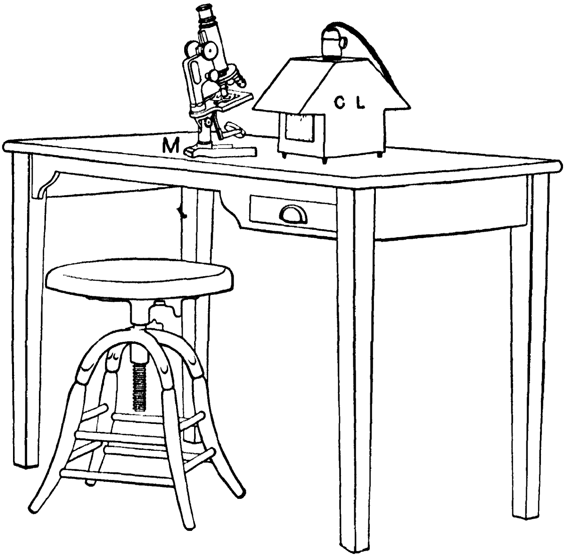


FIG. 53. LABORATORY TABLE, STOOL, MICROSCOPE AND CHALET LAMP WITH DAYLIGHT GLASS.

(About one-fifteenth natural size.)

CL Chalet microscope lamp with two windows of daylight glass on opposite sides under the overhanging roof. The roof serves to protect the eyes (fig. 46).

M Laboratory microscope, slightly inclined.

It will be noted that the table rail is cut out in front to avoid interference with the knees of the observer. A table drawer at the right can be pulled out without moving. The revolving piano stool can be adjusted to any desired height.

Condensers for transmitted light (§ 70) are complex optical appliances, sometimes almost as complex as objectives.

The student might fairly ask: Why be bothered with anything more than a mirror for lighting translucent objects. A glance at fig. 54 will show him that with a mirror only a narrow cone of light can be sent to the object. He will find in actual work that for powers of 8 (20x), 4 (40x), and 2 (90x) mm. the object cannot be lighted with a sufficient angle, or aperture, as it is now called, to bring out the details of structure that he is trying to see and understand.

If anything is certain in vision, it is that the details which can be made out clearly depend upon the aperture of the light from the object to the eye. If that be true, then it is essential that the object be supplied with light at an aperture great enough to furnish the required aperture of light for the eye.

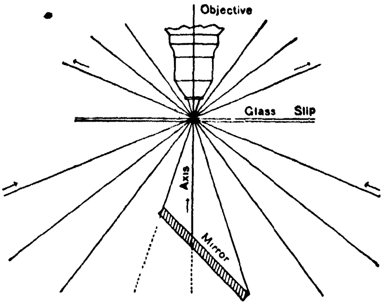


FIG. 54. DIAGRAM FROM BECK TO SHOW THE APERTURE REFLECTED BY THE MICROSCOPE MIRROR. IT IS ONLY ABOUT 0.25 N.A.

The diagram represents the light originating from a white surface or from the sky, and the small part which the microscope mirror can receive and reflect.

With opaque objects like snow, white paper, etc., the reflections are in all directions and if the light by which they are illuminated is brilliant enough, any aperture up to 1.00 N.A. will be satisfied if there is air between the white surface and the microscopic objective. If a greater aperture than 1.00 N.A. is required, as for immersion objectives, then the proper immersion liquid must be between the object and the front of the objective.

It is sometimes stated that if one points the microscope toward the sky out of doors, the aperture of any microscope objective will be filled with light. This is true for dry objectives which never require an aperture greater than 1.00 N.A. An immersion objective with an aperture above 1.00 N.A. would not be filled even from the sky, as can be seen easily by trying the experiment (§ 124).

§ 115. Angular and numerical aperture in microscopy. — By angular aperture is meant the angle in air formed by the border rays of the light passing from the object into the front lens of the objective (fig. 116).

If the light entering and leaving a lens or lens system were always from and into air, angular aperture would be entirely adequate; but as the light in immersion instruments (objectives and condensers) is from or into a medium with greater refractive index than air, the cone of light is modified by the refractive action of the medium which it traverses, hence the index of refraction of the medium in which the light cone passes must be considered. Abbe devised an expression which meets the needs. It is called Numerical Aperture (N.A.), and is found by multiplying the sine of the semi-angle of the cone of light in the medium by the refractive index of the medium. Stated mathematically it is: N.A. $= n \sin u$. In which n stands for the refractive index, and $\sin u$ for the natural sine of half the angle of the light in the medium.

The following generalizations can be made:

(*a*) With two media in contact, the sines of the angles of the rays of light in the two media are in inverse ratio to the index of refraction in the two media; consequently, the greater the difference in refraction, the greater the difference of the angle of the same light in the two media. Knowing any three of the factors, the fourth is readily found.

(*b*) The numerical aperture (N.A.) of the light passing from one medium directly into another remains constant, no matter how great the change in its angle.

(*c*) The numerical aperture (N.A.) of the light in any medium is the sine of the semi-angle of the light multiplied by the index of refraction of the medium (N.A. $= n \sin u$). Knowing any two of the factors, the third is readily determined.

By referring to fig. 116 and fig. 55, one can get a graphic view of the significance of these terms.

In fig. 55 *A*, the angle of the light in the air is 180° and the sine of half this angle is 1.00 ($\sin 90^\circ = 1.00$). If now this 180° of light in air passes directly into glass with an index of refraction of 1.52, its angle will be reduced from 180° to 82°, but its numerical aperture (N.A. $= n \sin u$) is not changed, and is 1.00 in both air and glass.

Suppose the light is passing from the glass into the air, as most frequently happens with condensers, then the light cone of 82° will expand into 180° or the whole hemisphere in the air. But the numerical aperture (N.A.1) remains unchanged as before.

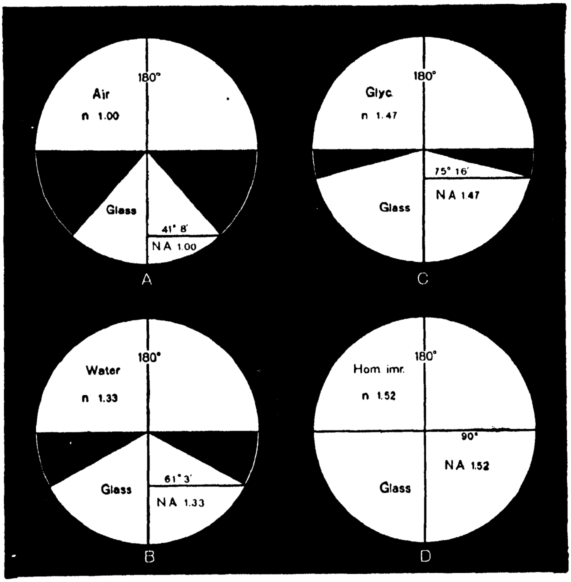


FIG. 55. DIAGRAMS TO SHOW THE ANGLE OF LIGHT IN GLASS REQUIRED TO FILL AN OVERLYING HEMISPHERE OF AIR, WATER, GLYCERIN OR HOMOGENEOUS LIQUID WITH LIGHT.

The diagrams show that in each case the angle of light required in the glass represents a numerical aperture equal to the refractive index (*n*) of the overlying medium. The dark parts of the hemispheres in *A*, *B* and *C* represent the segments not lighted. In *D* the whole sphere is lighted. Any light in excess of this aperture is reflected back into the condenser.

In fig. 55 *D* where there is glass below and homogeneous liquid above, the angle of the light is 180° in both and the numerical aperture is 1.52, agreeing with the refractive index.

§ **116. How to tell the part of the aperture filled with light.** — When an objective is focused upon any object one can tell the aperture of the objective being used by taking out the ocular and looking

Table Showing the Angle of Light in Different Media for the same Numerical Aperture with Dry, Water Immersion, and Homogeneous Immersion Objectives or Condensers.

(From the Journal of the Royal Microscopical Society.)

Numerical Aperture ($n \sin u$ = N.A.)	Angle in Air ($n = 1$)	Angle in Water ($n = 1.33$)	Angle in Homogeneous Media ($n = 1.52$)
1.52			180°
1.33		180°	122°6′
1.00	180°	97°31′	82°17′
0.76	98°56′	69°42′	60°
0.50	60°	44°10′	38°24′
0.25	28°57′	21°32′	18°56′

down the tube. The bright spot seen is the back lens of the objective. If it is all lighted, then the entire aperture is filled. If there is a bright spot in the middle and a dark rim around the edge, then it is but partly filled. For experiment use a transparent object like a stage micrometer or a very thin, lightly colored section. Use the 16 mm. (10x) objective. Focus the object. Then take out the ocular and look down the tube. Probably the whole of the back lens will be lighted. Close the iris diaphragm slowly and the margin of the back lens will have a dark rim around it as the iris gets so small that the aperture is not filled. Turn the 4 mm. (40x) objective in place and repeat the experiment. It is to be noted that the iris must be wider open for the 4 mm. (40x); and wider still for the 3 mm. (60x) (fig. 56). One can determine more easily and accurately the amount of aperture

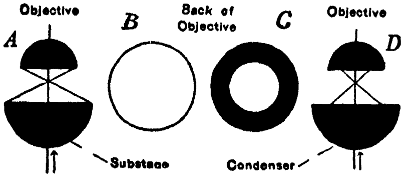


FIG. 56. APERTURE OF THE SUBSTAGE CONDENSER AND OF THE OBJECTIVE.

(From Nelson, Jour. Roy. Micr. Soc.)

A The cone of light from the condenser fills the aperture of the objective (*B*).

D The cone of light of the condenser only partly fills the aperture of the objective (*C*).

In *A* and *D* the condenser and objective are shown in section; in *B* and *C*, the back lens of the objectives is shown in face view as when looking down upon it with the ocular removed.

filled in the objective, the centering of the condenser and the lighting if a central pinhole cap is placed over the top of the tube and one looks down through the pinhole (fig. 58).

§ 117. Aperture; and centering the condenser by the eyepoint. — As stated by A. E. Wright, the part of the aperture of an objective lighted in any given case is most easily and accurately determined in the focused microscope by examining the eyepoint with a magnifier. One of the aplanats of 10 to 15 magnification is good for this. The magnifier can be held in the hand, by a lens holder or by Beck's lens holder for the eyepoint (figs. 24, 57). If the focused microscope is brilliantly lighted, the eyepoint can be seen and its position determined by the use of a piece of thin paper or by the use of ground glass, § 99. If the magnifier is held above this point and raised and lowered the eyepoint can be focused as if it were an object. When in focus one will see an image of the back lens of the objective and of the diaphragm opening. Open and close the iris and change the focus of the magnifier if necessary to make the diaphragm opening sharp. By closing the diaphragm the back lens of the objective will be only partly lighted. With low powers it is easy to open the iris wide enough to light the entire back lens. It will be seen in this experiment that the higher the power the wider open must be the iris to fill the back lens with light. This means that the higher power has a larger aperture and hence must be lighted by a wider aperture from the condenser; and that necessitates a wider opening to the iris. By looking at the eyepoint with the magnifier one can tell exactly how much of the back lens is lighted. That can be determined less certainly by taking out the ocular and looking down the tube of the microscope, or by using the pinhole cap (fig. 58).

As pointed out by Wright, p. 93, a study of the eyepoint by means of the lens (fig. 57) or pinhole cap (fig. 58) gives very definite information:

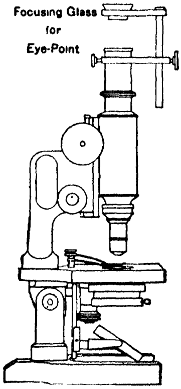


FIG. 57. COMPOUND MICROSCOPE WITH BECK'S ADJUSTABLE ARRANGEMENT FOR FOCUSING THE EYE-POINT.

(1) Whether the back lens of the objective is filled with light, or how nearly filled. One can then judge whether the diaphragm is opened the right amount.

(2) Whether the condenser axis is centered to the axis of the objective. If it is not the opening of the diaphragm will be closer to one side. If centered the diaphragm opening will be in the center of the back lens of the objective.

(3) Dust or other opacities on the back lens will be brought out.

(4) The presence of air bubbles in the immersion liquid will appear.

§ **118. Experiments in centering.** — Use a transparent or very translucent specimen, and focus the microscope with any objective. It is better to begin with an 8 mm. (20x) or a 16 mm. (10x) objective.

Close the iris as much as possible, and then examine the eyepoint with the magnifier. Or use the pinhole cap in place of the ocular (fig. 58). The opening of the iris should appear in the middle of the back lens of the objective. Open the iris slowly while looking into the magnifier, and note whether as the iris opens, it disappears around the edge of the back lens uniformly or whether it remains in sight longer on one side. If the small bright spot seems to be in the center of the back lens, and the iris disappears all around the back lens at the same time, then the condenser is centered. If the small opening is eccentric, and if the diaphragm does not disappear all at once on opening the iris, then the condenser and objective are not centered, that is, are not on the same optic axis.

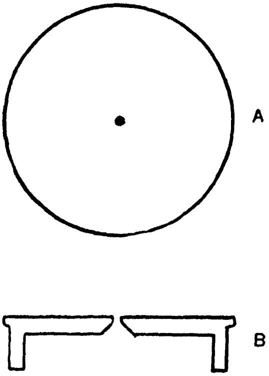


FIG. 58. PINHOLE CAP TO AID IN CENTERING AND DETERMINING APERTURE.

A Top view.
B Sectional view.

If they are not centered, and the substage fitting has centering screws (fig. 60) these should be turned with the two hands while looking into the magnifier until the small opening seems central, and then until the iris disappears equally all around the border of the back lens.

If there are no centering screws to the substage, as in student microscopes and some others, one can ignore the fact if the eccentricity is slight, or if the work is not too exacting. If the eccentricity is great, it is best to send the microscope to the makers for centering, unless the user has the knowledge and mechanical skill to make the corrections himself.

With some microscopes the iris diaphragm is so placed that the condenser projects a real image of it above the stage. If one closes the iris and uses a 16 (10x) or 8 (20x) mm. objective, this real image of the iris can be seen in the field by focusing up from the position where a specimen would be in focus. In some cases one might need to focus down slightly. If the image of the iris opening is in the middle of the field, the condenser is centered to the objective; if it is not in the middle, one can center by using the centering screws (fig. 60).

Sometimes also the iris can be made eccentric by means of a milled wheel, therefore before trying the centering, one must be sure that the iris diaphragm is centered, and not eccentric to the condenser.

§ **119. Experiment with the sky as light source.** — Remove the body of the microscope with its objectives in the nose-piece from the microscope stand. Put some lens paper in the tube and insert the ocular firmly. This is so it will not fall out when the tube is pointed to the sky. Take along the magnifier to look at the eyepoint or use its pinhole cap in place of an ocular (§ 117). Go out in the open and point the microscope up at the sky. Use the objectives in turn. Each of the dry ones will have its aperture completely filled; but the immersion objective if of over 1.00 N.A. will not be filled. There will be a dark rim around the objective representing the aperture above 1.00 N.A.

§ **120. Aperture filled by a white opaque object.** — Put a white card or any thick piece of white paper on the stage of the microscope. Put the microscope in a window where the sun can shine on the paper, or use some brilliant artificial light and focus on the upper surface of the paper under the objective. The daylight lamp (figs.

79 or 80) for dark-field work is excellent for this experiment. The success of the experiment depends upon a bright light; how it is obtained is not important.

Focus first the 16 mm. (10x) on the top of the card. The back lens will be full of light showing that the whole aperture is satisfied. The same will be found for the 4 mm. (40x). Now turn the homogeneous immersion objective with an aperture of 1.25 to 1.40 in place and focus it down almost to the paper. The back lens will now have a dark rim around it.

To satisfy the aperture completely some homogeneous liquid must be put between the paper and the front lens of the objective. Keep the eye over the magnifier above the eyepoint or use a pinhole cap and with a small brush or in some other way put some homogeneous liquid on the paper at the edge of the objective. It will run under by capillarity; and as it spreads over the face of the objective, the dark rim will disappear. Prove this is true by focusing up until the homogeneous immersion is broken. The dark rim will reappear. Now focus down until the objective again becomes immersed and the whole aperture will again be filled with light. In a word: These experiments show that a numerical aperture not exceeding 1.00 N.A. can be supplied to the microscope by light in air.

§ **121. Aperture with transmitted light from a mirror.** — With transmitted light through thin white paper or ground glass or other light scattering substance, the entire hemisphere will also be filled with light by the mirror. This is easily demonstrated by placing a piece of glass ground on both surfaces on the stage of the microscope, and lighting it well with the plain mirror. If any dry objective is focused on the upper granular surface of the glass, its aperture will be found entirely filled with light. If now a homogeneous immersion objective is focused on the granular surface, its aperture will be only partly filled with light. Just as when pointing the objective toward the open sky, the central part of the back lens will be lighted, but there will be a dark border all around it. This border may be dimly lighted by the diffracted light, but it will not be anywhere near as light as the central 1.00 N.A. Now while looking at the image of the back lens in the eyepoint (§ 117), if some homogeneous immersion liquid is put on the glass at the edge of the objective, it will gradually run in between the objective front and the glass and as it does so, one can see the whole aperture becoming filled. If the objective is focused up until the immersion liquid breaks, leaving air again between the ground glass and the objective front, the aperture will again be filled only to the 1.00 N.A. as shown by the dark border.

If rather thick cedar oil is used for the immersion liquid, it is possible by focusing up carefully to have the oil column only partly uncover the front lens of the objective, then the part uncovered will show the dark rim of unfilled aperture while the other part will be completely filled. By careful focusing one can make the immersion liquid flow back and forth over the front lens and see the change in aperture from the covered to the uncovered portion. After such an experiment one can never again doubt the efficiency of an immersion fluid of greater refraction than air for increasing the aperture of the light.

§ 122. **Aperture with translucent objects.** — If the object does not scatter the light as with ground glass or thin paper, etc., then the aperture of the light going to the object will pass on to the objective and will determine the amount of aperture the objective has available for forming an image.

Aperture with translucent objects lighted by a mirror. — Use any translucent or transparent object like a stage micrometer or a thin histological section. Turn the condenser aside, and use the mirror only for illumination. Use first a 16 mm. (10x) objective. Focus the well lighted specimen and examine the back lens by the pinhole cap after the ocular has been removed, or by looking at the eyepoint with a magnifier (§ 117). With a 16 mm. (10x) of N.A. 0.25, the entire aperture will be filled with light either from the plane or from the concave mirror. With a 16 mm. (10x) objective of 0.30 N.A. the 5 centimeter mirror barely fills the aperture. A mirror of 6 centimeters diameter fills the aperture completely. As the usual microscope mirror is rarely over 5 centimeters in diameter, it can be stated that with a mirror alone only about 0.25 N.A. can be supplied.

§ 123. **Aperture with translucent objects lighted by a substage**

condenser. — Use a translucent object mounted in Canada balsam, but put the condenser in position and close to the object. Light with the plane mirror. First use the 16 mm. (10x) objective; open the iris. Look down the tube or at the eyepoint. The aperture is completely filled. Turn a 4 mm. (40x) objective into position, focus, and then look down the tube or at the eyepoint with a magnifier. Open and close the iris. By opening it widely the entire aperture will be used as shown by the back lens full of light.

Now turn the homogeneous immersion into position, and without adding any immersion liquid, focus the objective. Look at the back lens and it will be found only partly filled with light, although the iris is wide open. Add the homogeneous liquid so that there will be immersion contact with the objective and specimen. Look at the back lens again, and it will be found that the aperture is more completely filled, but there is still a dark rim around the outside just as there was when looking at the sky (§ 119). This is in spite of the fact that the immersion objective is in homogeneous immersion contact with the object or its cover-glass. In § 120 when the homogeneous liquid made immersion contact, the aperture was completely filled with light; why is it not in this experiment? With §§ 120–121 the object itself scattered the light and filled the whole hemisphere above, consequently when the homogeneous liquid was added, the full aperture could be satisfied. In this case the specimen does not scatter the light to any extent, and the objective receives only the aperture of light that went from the condenser through the object to the objective.

But as the substage condenser has an aperture of 1.20 to 1.40 N.A., why does it not supply the adequate aperture to the object so that the objective aperture will be completely satisfied? The law of optics by which no aperture greater than 1.00 N.A. can pass from a denser medium to air prevails here, for there is air between the top of the condenser and the under surface of the glass slip bearing the specimen, hence the object cannot be lighted by such an arrangement with a cone with an aperture greater than 1.00 N.A. The way the difficulty is overcome is shown in the next section.

§ 124. Immersion substage condenser. — If the substage condenser has air between its upper face and the under surface of the glass slip on which the specimen is mounted, the condenser, no matter what its possible aperture, can send into the air only an aperture of 1.00 N.A. (fig. 55 *A*). If the condenser is to illuminate the object with an aperture greater than one, then there must be a medium of higher refractive index than air between the top of the condenser and the under surface of the glass slip.

This statement is very easily verified by the following experiment. Use a homogeneous immersion objective, and some rather transparent object, or take a clean glass slip and with a fine pen make a line in the middle with India ink. This is to give something to focus on. Use the homogeneous immersion fluid on the objective as usual and focus the ink line, then move the slip so that the black line will not obscure the field. Open the iris diaphragm to its full extent so that the condenser will have a chance to work at full aperture. Light well with the plane mirror. Examine the eyepoint with a magnifier (§ 117) or use the pinhole cap (fig. 58) and note that there is a dark rim around the margin of the back lens of the objective just as there was when the microscope was pointed toward the sky or used with the ground glass. That is, as only a numerical aperture of 1.00 could be supplied by the condenser with air between it and the glass slip, only a numerical aperture of 1.00 of the objective is filled. Without changing the objective in any way, lower the condenser and put on its upper face a good drop of the immersion liquid, and then slowly run it up until the oil on its upper face comes in contact with the lower face of the slip. Now look at the back lens of the objective and note that the aperture of the objective is filled. To make still more striking the demonstration, while looking at the back lens of the objective, lower the condenser very slightly. This will break the immersion contact on one side. Lower till about half of the aperture shows immersion contact. On the side where the immersion is lost the dark rim will show, and on the side still immersed it will be absent (§§ 120–121).

If then one is to use the full aperture of any objective with an aperture greater than one 1.00 N.A., both the objective and the condenser must be immersed in some fluid with a higher refractive index than air. In the experiment the homogeneous liquid was of much higher index (1.52).

§ 125. Correlation of objective and condenser aperture. — It is evident from the experiment in § 124 that if the objective is to be used at its full aperture the condenser on the microscope must be of sufficient aperture to satisfy the objective. Strange as it may appear, however, many microscopes are regularly supplied with objectives of 1.25 to 1.30 N.A., and much is made of the fact, but the condenser supplied on the microscope has at the outside not over 1.20 N.A. and not one in a hundred who uses such a microscope ever makes immersion contact with the condenser and glass slip, and therefore never uses more than an aperture of 1.00 N.A. The few that would like to utilize the high aperture, of which so much is said in the microscope catalogues of all manufacturers, cannot do so with the low apertured condenser regularly supplied.

Fortunately it is now becoming the fashion of users of the microscope to know something of the optics of their most efficient servant and the manufacturers are helping to spread the knowledge needed, and to supply most excellent condensers to meet the most exacting requirements. It is to the credit of the British microscopists and manufacturers that they have always been in the lead in such matters. — (See Brewster, Carpenter-Dallinger, Nelson, Coles, and Conrad Beck, etc.)

§ 126. Experiment with a 1.20 N.A. condenser (fig. 59) and an objective with 1.25 to 1.40 N.A. — Use a transparent specimen in Canada balsam like that used in § 125. Connect condenser and glass slip with homogeneous liquid (§ 125). Use the homogeneous immersion objective with as high an aperture as is available, and employ the correct homogeneous liquid. Focus sharply. Now look at the back lens of the objective and open and close the iris diaphragm so that the opening shows unmistakably. Now open the iris to its full extent. There will still be a dark rim around the outside. That is, a condenser with an aperture of 1.20 cannot supply an aperture of 1.25 to 1.40 N.A. even though it is in immersion contact with the under side of the slip.

If one has available a substage condenser of 1.40 N.A. and it is in immersion contact with the glass slip, the full aperture of the objective can be filled. For the methods of determining the numerical aperture (N.A.) of objectives and condensers see § 261 et. sq.

§ **127. Diffracted light and aperture.** — With many objects when illuminated by transmitted light there is considerable, and with all objects a certain amount of diffraction of the light going through them; consequently there is a greater or less amount of diffracted light in addition to the original cone. This additional diffracted light may be so great in amount that the whole aperture of the objective of 1.30 to 1.40 N.A. may be filled, although the condenser may have an aperture of only 1.20 N.A. In most cases the diffracted light is very weak as compared with that directly from the condenser and object, hence the aperture supplied by the condenser will be very bright in comparison with the aperture filled by the diffracted light, and the zone at the edge of the objective appears as a gray rim around the edge. If there were no diffracted light, it would look black, beyond the aperture supplied by the condenser.

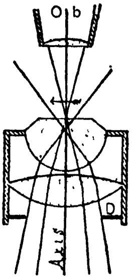


FIG. 59. ABBE CONDENSER WITH CONVERGING LIGHT. *ob* Objective; *D* Iris diaphragm.

Much has been said in recent years about the supreme importance of diffracted light in microscopy. As diffracted light forms an important part of all light, no one can doubt that it is of importance. It seems to the writer, however, that this constituent has been by some over-emphasized and given undue prominence in microscopic vision.

§ **128. Optical corrections of the substage condenser.** — Fundamentally the substage condenser has for its purpose the illumination of the object. One of the pictures showing the use of a condenser for the microscope is given by Descartes (fig. 312) and is a plano-convex lens with the plane side toward the source of light.

In the early period of uncorrected lenses, and also at the present time with the popular Abbe condenser, which is neither achromatic nor aplanatic, the main purpose seems to be to supply an abundance of light. If one bears in mind that no matter how carefully corrected the objective may be, if the light received by it from the condenser is full of chromatic and spherical aberration, it is not fair to expect the objective to make a perfect picture. Over 100 years ago Sir David Brewster appreciated the difficulties, and stated with great clearness what seemed to him the means for obtaining correct microscopic images. He says of illumination in general: "The art of illuminating microscopic objects is not of less importance than that of preparing them for observation." "The eye should be protected from all extraneous light, and should not receive any of the light which proceeds from the illuminating center, excepting that portion of it which is transmitted through or reflected from the object." So likewise the value and character of the substage condenser was thoroughly understood and pointed out by him as follows: "I have no hesitation in saying that the apparatus for illumination requires to be as perfect as the apparatus for vision, and on this account I would recommend that the illuminating lens should be perfectly free of chromatic and spherical aberration, and the greatest care be taken to exclude all extraneous light both from the object and from the eye of the observer." (See Sir David Brewster's treatise on the Microscope, 1837, pp. 136, 138, 146, and the Edinburgh Journal of Science, new series, No. 11 (1831) p. 83.)

While the simple and relatively inexpensive substage condensers of the Abbe type (fig. 59) serve fairly well for student and general work it is evident that for the most exacting work achromatic-aplanatic condensers are required. If it is true, as all agree, that for a perfect image of an object no light should reach the eye except from the object, then it is readily seen that an achromatic-aplanatic condenser must be used for it is only by such a condenser that the object can be so lighted that no light reaches the eye except from the object. Condensers with both chromatic and spherical aberration cannot direct their entire cone of light upon the object and hence much light must reach the eye which does not come from the object. In so far as that is the case, the image will be imperfect.

The clearness of the images with the best dark-field condensers gives abundant evidence for this contention. With the highest powers, if perfection is sought for, the object must be mounted on glass of cover-glass thickness, and a homogeneous immersion objective used for condenser as well as for forming the microscopic image, and both are in homogeneous contact below and above the object respectively. Or a condenser of objective perfection must be used. Special homogeneous immersion condensers are demanded by some workers, and are willingly produced by the opticians. However, the common practice of using the same substage condenser, dry or immersed, as occasion requires, is justified from the fact that the working distance of the substage condenser is exceedingly short, and hence its spherical and chromatic corrections are very slightly affected whether used dry or immersed. Of course, if an aperture greater than 1.00 N.A. is desired the condenser must be immersed. (§§ 124–125.)

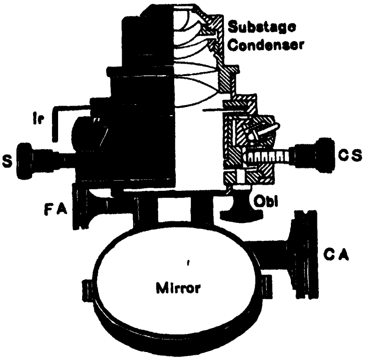


FIG. 60. ACHROMATIC-APLANATIC, CENTERING, SUBSTAGE CONDENSER. (From the Catalogue of the Spencer Lens Co.)

CA Coarse adjustment for the condenser.

FA Fine adjustment for the condenser.

CS, *CS* Centering screws for centering the condenser.

Ir Handle for working the iris diaphragm.

Obl Milled head for working the oblique-light iris.

§ **128a.** The writer is indebted to Mr. H. N. Ott of the Spencer Lens Company for help in this matter. Mr. Ott says: "If the object were in optical contact with the objective, that is, the working distance reduced to zero, and the condenser in like manner in optical contact, there would be no difference whether the objective or the condenser were immersed or not. The working distance of the objective, small as it is, is relatively great as compared with the working distance of the condenser. The working distance of the condenser approaches so nearly to zero that there is no great difference in its chromatic and spherical corrections whether it is immersed or not. Hence all forms of condensers, the Abbe type and the aplanatic-achromatic type, may be used either immersed or not as required. It is of course impossible to get an aperture greater than 1.00 N.A. unless the condenser is immersed."

§ **129. Condensers for student microscopes.** — These are usually of the non-achromatic or Abbe form (fig. 61); and the construction of the modern microscope is sufficiently accurate so that they are nearly enough centered for all practical purposes (Beck). Sometimes they get so badly decentered that they should be either sent to the makers, or should be put right by some one with mechanical ability connected with the laboratory.

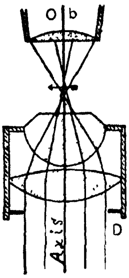


Fig. 61. Abbe Condenser with Parallel Light.

Ob Objective; *D* Iris diaphragm.

§ **130. Mirror and light for the condenser.** — It is best to use light with parallel rays. The rays of daylight are practically parallel; it is best, therefore, to employ the plane mirror for all but the lowest powers. If low powers are used, the whole field is not illuminated with the plane mirror when the condenser is close to the object; furthermore, the image of the window frame, objects outside the building, as trees, etc., would appear with unpleasant distinctness in the field of the microscope. To overcome these defects one can lower the condenser and thus light the object with a diverging cone of light, or use the concave mirror and attain the same end when the condenser is close to the object (fig. 57).

§ **131. Lighting the entire field with a condenser.** — With the condenser there are two conditions that must be fulfilled; the proper aperture must be used, and the whole field must be lighted. As seen in § 124 the diaphragm of the condenser regulates the aperture of the illuminating cone but does not affect the size of the lighted field unless it is far below the condenser. The size of field that is lighted by a condenser can be modified in two ways:

(1) Suppose that the image of the source of light is focused on the object, the size of that image will determine the size of field which is illuminated in a given case. If the illuminated field is not so large as the objective field, then the source of light is too small, or too far away. In that case, use a larger source or bring the source closer to the microscope.

(2) By lowering the condenser or using the concave mirror a much larger object can be fully lighted, as it is in a diverging cone

of light above the focal point of the condenser where the light is spread over a greater area (fig. 61).

For quite low objectives, 35 (4.5x) to 60 mm. (2.6x) focus, it is better to remove the condenser and use the mirror only. The whole field can be illuminated easily and sufficiently in this way.

§ 132. Substage condensers with removable top. — In most of the good modern, substage condensers the upper or top element is removable, and the element next the mirror used alone. With this lower element a sufficiently large field can be lighted to satisfy nearly all requirements. Indeed the lower element is so well corrected that for most purposes objectives as high as the 4 mm. (40x) are well lighted, provided the condenser is placed at the right distance below the stage. One can easily determine that by trial. As the lower element is of much longer focus than the entire condenser, it must be lowered. For the dark-field element to go with these separable condensers see § 181.

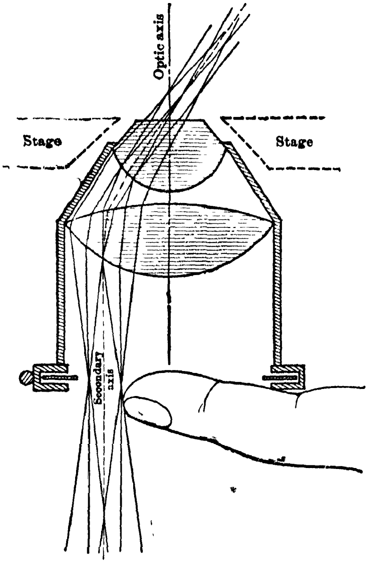


FIG. 62. OBLIQUE LIGHT WITH A CONDENSER. (From Chamot).

The iris diaphragm is opened completely and the light from one side is blocked out by inserting the finger; this gives unsymmetrical light and all of it is oblique to the optic axis.

§ 133. Axial and oblique light with the condenser. — To demonstrate the effect of the methods of illumination when a condenser is used, take any striking preparation like a diatom (*Pleurosigma angulatum*, for example); employ a 4 mm. (40x) objective. Being sure that the condenser is centered, fill the aperture of the objective about ¾ full of light (§ 124). Study the preparation with the central light and note the appearance of the markings. Cover a part of the diaphragm opening by putting the finger or some other opaque object between it and the mirror (fig. 62). Note that the markings come out more strongly. Hold the finger in position and open the diaphragm widely and see if the markings can still be made out. Now remove the finger so that the object is lighted by the full aperture of central light. Probably the markings will not appear at all. Put the finger back in position to give oblique light and the markings will again be seen. Remove the finger and slowly close up the diaphragm. When the proper aperture is reached the markings will again appear.

For histological preparations the oblique light is not a help in bringing out details of structure. There the end is reached by using the proper aperture, regulating the source of light, and by differential staining.

§ **134. Lateral swaying.** — Frequently in studying an object, especially with a high power, the image will appear to sway from side to side in focusing up or down. A glass stage micrometer or fly's wing is an excellent object. Make the light central or axial, focus up and down, and notice that the lines simply disappear or grow dim. Now make the light oblique, either by making the diaphragm opening eccentric or, if simply a mirror is used, by swinging the mirror sidewise. On focusing up and down, the lines will sway from side to side. What is the direction of apparent movement in focusing down with reference to the illuminating ray? What in focusing up? If one understands the experiment it may sometimes save a great deal of confusion.

§ **135. Critical illumination.** — This expression strictly used means that the image of the source of illumination is projected upon the object by the condenser (fig. 63). The object then appears in the image of the light source. If the image of the light source is to be used with the chalet lamp, then the daylight glass filter must be polished on both sides and the bulb be of transparent, not frosted glass. The filament is not large enough to cover the field except when using high objectives.

Used less strictly it means any very exact method of illumination which will give the clearest image in any given case. Many good workers get a sharp image of the source of light upon the object and then focus the condenser down just enough to throw the image out of focus. If one uses ground daylight glass or ground ordinary glass over the window of the chalet lamp for example, the ground glass becomes the source of light and if that is focused on the specimen, the granulation of the surface takes away from the sharpness. This is entirely obviated by throwing the image of the ground glass just out of focus. A lamp flame in like manner, or the coils of an incandescent lamp have irregularities that injure the microscopic image if the image of the light source is sharply focused on the object. Unless, then, the light source is entirely homogeneous, it is better to have its image out of focus on the object.

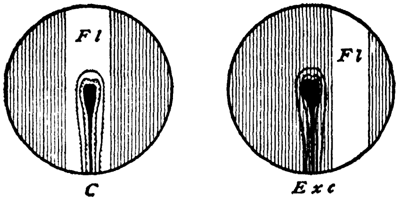


Fig. 63. Field of the Microscope Showing the Light in the Center and to One Side.

C, Fl The light is in the center and illuminates the object.

Exc, Fl The light is at one side of the center and does not illuminate the object. (The field is not fully lighted, as a low power is used to center the object and the light.)

§ **136. Aperture of the condenser with critical illumination.** — The most general rule followed — the Nelsonian rule — is to open the iris diaphragm of the condenser till about three-fourths of the aperture of the objective is filled with light. For moderate powers one can tell easily by taking out the ocular and looking down the tube of the microscope at the back lens of the objective. About three-fourths of the lens should be lighted. One can judge by opening and closing the iris until it is judged that three-fourths the diameter is bright, and one-fourth dark. For high powers one can tell more accurately by looking with a magnifier or the pinhole cap (§ 118, fig. 58) (figs. 57–58, § 117) at the eyepoint above the ocular when the microscope is in focus. In the actual study the diaphragm is frequently closed more or less and opened to get the best effect in any given case. One has to keep in mind always, however, that the amount of fine detail that it is possible to see depends upon the numerical aperture by which it is studied. As will appear in the next section the amount of aperture which is usable in a given case depends partly on the size of the source of light, and the character of that light.

§ 137. The source and character of the light. — The statements of three British workers are so to the point in this connection that they are quoted: — Sir David Brewster, 1831–1837. "The greatest care (should) be taken to exclude all extraneous light both from the object and from the eye of the observer." Wright, "Principles of Microscopy," p. 219: "The necessity for the regulation of the source of illumination will appear when we consider the optical conditions which obtain where an extended radiant field such as is furnished by the sky or a broad lamp flame is employed as a source of light. With such an extended source, its image will be larger than the field of any objective..

From the radiant points included within this illuminated area beams will pass into the aperture of the objective. Those from the center of the field — always assuming that their numerical aperture does not exceed the numerical aperture of the objective — will pass through the aperture unmutilated. It will be different with respect to the beams which proceed from the periphery of the field. These, taking the aperture obliquely, will, unless in the case where their numerical aperture is much less than that of the objective, be cut down in an unsymmetrical manner by the margin of the objective, exactly in the same way as would be the case if transmitted through an elliptical, or, in the extreme case, through a slit aperture.

"It follows that while the radiant points in the center of the field will be represented in the image by circular antipoints whose dimensions will be determined by the full numerical aperture of the objective, the radiant points on the periphery of the field will be represented in the image by elliptical or linear antipoints whose long axes will in each case be disposed radially to the aperture, overlapping the antipoints in the center of the field in such a manner as to fog the image."

Conrad Beck, Journal of the Royal Microscopical Society, 1922, pp. 399–405, and in his book "The Microscope," Part II, 1924, p. 105: "For the correct delineation of a microscopic object seen with transmitted light, no light should reach the eye that has not passed through the object." It is well also to keep in mind what was found out by the older opticians, viz., that for the discrimination of the fine details of an object one is far more successful when working in a dark or a dimly lighted room so that the light from the object under the microscope or seen through any optical instrument is the only light entering the eye. The astronomers also found long ago that no successful study could be made of the faint nebulae on a bright moonlight night.

§ 138. Brightness for the best visual acuity. — For the best visual acuity with the naked eye it is believed that light objects should be illuminated by 1- to 3-foot (10- to 30-meter) candles. Dark objects must have a proportionally increased illumination.

As the microscope gives an apparent increase in the area of the object looked at, i.e., in proportion to the square of the magnifying power, it follows that if the same brilliancy is to be maintained the light must be increased in the same proportion. That is, if a square centimeter of an opaque object had a given brilliancy when seen by the naked eye, when magnified 10 diameters giving an area of 100 square centimeters, the illumination must be increased 100 times if the brightness remains constant.

For translucent objects lighted by transmitted light, the matter is complicated because microscopic objectives vary in aperture from the lower to the higher powers regularly, and with the same powers depending on their construction, also on the aperture of the cone of light sent to the object by the mirror or through the condenser. As the brilliancy varies directly as the square of the numerical aperture, but inversely as the square of the magnifying power, both the aperture and the magnifying power must be considered when estimating the intensity of the light required to maintain a constant favorable brilliancy of the object for the discrimination of its fine details.

Conrad Beck in his recent work on "The Microscope," Part II, p. 104, taking into account the aperture, the magnifying power and the light losses in the microscope, gives the following table of desirable foot candles for different powers. Candle meters in round numbers have been added in the third column.

Mag. power	*Foot candles*	*Candle meters*
50	16	172
100	20	255
300	50	538
600	80	861
1000	250	2691
2000	1050	11302

Diffuse daylight can rarely supply more than 100 foot candles (1076 candle meters) and hence is not sufficiently brilliant for powers above 500 to 600 diameters. Besides it is exceedingly variable in intensity during the different hours of daylight, and in different seasons of the year. For these reasons many workers discard daylight altogether for exacting work, and utilize some artificial source like one of the different daylight lamps (figs. 46, 80), the "tungsarc" or the "pointlite" lamp. Even the petroleum or kerosene lamp has its enthusiastic advocates where color values are not involved. From personal observation the yellow petroleum light does not permit so clear an appearance of fine details as the daylight lamp. Furthermore it is much more tiring to the eye with most observers who have made comparative tests.

For some special work it has been found desirable to use color screens or even pure spectral colors. In general the shorter the wave length of the light used, the finer the detail that can be made out. For example, with rulings and diatoms, finer lines and finer markings can be seen with blue light than with red.

In recent times there has been a revival of the use of polarized light for the study of microscopic objects, and much is hoped from that source (§ 216). It is anticipated that the ultra-violet microscope will add even greater information (§ 303).

§ 139. Glare and fog in the microscope. — Even when one has a good microscope, the image may not be sharp and clear, but indistinct and hazy. This may be due to mist on some of the lenses. For example, on a cool morning, mist may collect on the ocular from the breath of the observer, or from the moisture emanating from the eye. No clear image can be seen through mist. Again, the dry objective may have been smeared with immersion liquid or with Canada balsam or other mounting substance. No clear image can be seen through dirty glass surfaces.

Although the microscope lenses may all be clean and of excellent quality there may also be glare and fog from the illumination. It is well also to keep in mind that the image may be greatly interfered with or even spoiled by the glare of an air bubble in the mounting medium near the object, or in the immersion fluid for the objective or the condenser. See Sir A. E. Wright, pp. 219–222.

§ **140. Experiments for glare and fog.** — Use a stage micrometer or some other very transparent specimen under a cover-glass in air. Put the microscope facing a window. Use a 16 mm. (10x) objective and a rather high eyepiece (10x to 25x). Have the condenser up close to the glass slip carrying the object. Put an ink spot or a faint mark with a glass pencil near the object so that it will be possible to focus easily. With the iris of the condenser nearly closed, focus the specimen. Then while looking into the microscope gradually open the iris and note the gradual dimming of the image. When the iris is wide open it will look as if a veil or mist were over the image, or the image may be wholly obliterated. This occurs because there is so much light around and near the object that gets into the microscope with the light from the object itself. One can prove that it is the light which is not from the object in this way: (1) Use a piece of dark, thick paper and make a hole about 10 millimeters in diameter in it. Hold this opening between the window and the mirror so that the light reaching the mirror comes only through the 10 millimeter opening. The outline of the object will again appear. It may be necessary to use a screen with even a smaller hole. Restricting the source of light cuts off the adventitious light which did not come from the object. (2) The second method is to eliminate the more oblique rays of the cone of light by closing the condenser iris to a greater or less extent. Remove the screen with the 10 mm. aperture and let the full light of the window strike the mirror. Then gradually close the iris of the condenser. The image will appear. One can make it appear or disappear by opening and closing the iris. In case the specimen is not so delicate as the lines of a stage micrometer ruled by a diamond, the specimen may not disappear entirely, but it will become much fainter when flooded with light by the full aperture, as many of the oblique rays enter the microscope that did not come from the object. In practice one really makes use of both the iris diaphragm and the regulation of the source of light for rendering the image distinct. However, if the finest details are to be seen the aperture must be retained. It is not to be forgotten, too, that an aperture above 1.00 N.A. cannot be obtained except by immersing the condenser (§ 124).

Fortunately objects mounted in Canada balsam, or other medium with the refractive index of glass, are not so subject to the disturbing effects of glare as when mounted in air or in media of low refractive index, hence one can use the full aperture of the objective most satisfactorily with balsam mounts.

As stated in § 136 Mr. Nelson makes the generalization that the best results are obtained in critical work by using about three-fourths the aperture of the objective. Conrad Beck contends that, with the proper source of light, one can use the full aperture; he also emphasizes the statement that to avoid the glare from the surrounding parts of the specimen while one should use the whole aperture of an objective, the iris of the condenser should be opened only enough just to fill the aperture.

The whole matter may be understood if one keeps in mind the fundamental principle that for the clearest vision only the light from the object should enter the microscope and form the image in the eye. In the first method there is the defect that the whole field may not be illuminated although the whole aperture of the objective may be filled. The second method restricts the aperture of the objective and hence in so far limits the resolving power of the microscope. By a judicious selection of the size of the source of light, and the correct intensity, and by regulating the aperture in each special case one can get the best results. This ability comes, like other good things, only by much practice even after the principles are understood.

§ 141. Microscopic objectives for research. — For the modern researcher there are three types of objectives to choose from: (*a*)

Achromatic objectives with lenses made wholly of glass. — With these the spherical aberration is corrected for one spectral color, the one selected being in the brightest part of the visible spectrum in the yellow green. For chromatic correlation, two colors only can be brought to one focus. While this statement might lead one to think that such objectives were very inferior optical instruments, in practice this is found not to be the case for the corrections being made in the part of the visible spectrum brightest to the eye, the defects in the outer, less brilliant parts (red and blue ends of the spectrum), while they exist, are overpowered, in the words of the older opticians, by the more brilliant part of the spectrum and hence do not obtrude themselves unduly.

(*b*) *Fluorite or semi-apochromatic objectives.* — This second group now coming largely into use for the more exacting work has added or substituted for one of the glass lenses in the combination, a lens of the natural mineral fluorite. This with its moderate refractive index and very small dispersion, makes it possible to construct objectives with more perfect corrections than the achromats. They are very good for photography.

(*c*) *Apochromatic objectives.* — These were so named by Abbe. Their characteristic is that spherical correction can be made with two colors, and chromatic correction for three colors of the visible spectrum. As it was next to impossible to make the full corrections in the higher power objectives, a special series of oculars were made to go with the apochromats and finish the corrections. These are for that reason called compensation oculars.

Besides the use of fluorite in apochromats and fluorite objectives, all modern objectives and other optical apparatus make use of new forms of glass, which is now made in America as well as in Europe. With these new forms of glass, and their range of refractive and dispersive power, it is now possible to render all optical instruments more perfect, even without the use of fluorite. For the most perfect results, however, fluorite must be used.

It is strongly advised that every one who is to use the microscope for research should become thoroughly familiar with experiments such as were devised by Abbe, and made possible with his test-plate. Without these experiments it would be almost impossible to believe that thickness of cover and length of the tube of the microscope could make such a difference in the clearness of the image seen with high objectives.

With this test-plate, too, one can get a most striking proof of the higher corrections of the apochromatic and fluorite objectives over even the best achromatic objectives. After such a clear demonstration as the test-plate affords, the researcher can never again feel that it is a waste of time to make sure that the optical apparatus is adjusted to enable it to give its best effects.

§ **142. Condensers for research.** — From what has been said in this chapter it must be evident that to get the full benefit of the best research objectives one must use with them a well corrected condenser with sufficient aperture; and furthermore it must be borne in mind that no condenser can pass on to the object an aperture greater than 1.00 N.A. unless the slide is connected with the condenser by means of a medium of sufficient refractive index (fig. 55).

§ **143. The Abbe Test-plate and the method of its use.** — This test-plate is intended for the examination of objectives with reference to their corrections for spherical and chromatic aberration and for estimating the thickness of the cover-glasses for which the spherical aberration is best corrected.

"The test-plate consists of a series of cover-glasses ranging in thickness from 0.09 mm. to 0.24 mm., silvered on the under surface and cemented side by side on a slide. The thickness of each is written on the silver film. Groups of parallel lines are cut through the film and these are so coarsely ruled that they are easily resolved by the lowest powers, yet from the extreme thinness of the silver they form a very delicate test for objectives of even the highest power and widest aperture. Full directions accompany the test-plate.

§ **144. Oculars to use.** — The Huygenian oculars with magnification of 2x to 10x answer very well for the achromatic and fluorite objectives of all powers, but for the apochromatic objectives oculars should be so constructed that they compensate for defects in the objectives. These so-called compensating oculars are also good for the high power modern achromatic and fluorite objectives, when high power oculars are to be employed. This is especially true, for dark-field microscopy, as is shown in Chapter III.

Besides the compensating oculars strictly so called, a great and successful effort has been made in the last few years to give the ocular a greater optical perfection by the use of combinations of lenses instead of the simple lenses of the Huygenian form. These go by various trade names as indicated in §43a.

§ 145. Oculars and spectacles. — As the eye of the observer is the last link in the optical chain forming a microscope, the perfection of the magnified image depends in part at least upon the perfection of the eye. As the seeing brain must gets its data from the retinal image, no argument is needed to show that this retinal image is of supreme importance. Indeed, the whole purpose of the entire microscope is to aid in making a perfect retinal image.

At present nearly every researcher must wear spectacles to correct dioptric eye defects such as astigmatism, etc. Fortunately the area of the eyepoint of the microscope is so small that only a small part of the cornea of the eye is involved, and the iris of the eye serves to cut out border rays that would make confusion. Nevertheless a defective optical part of the eye cannot give a perfect retinal image, and if the spectacles serve to make the retinal image more perfect it follows that the spectacles should be worn in exacting microscopic work. The difficulty in keeping the spectacles on in microscopic observation is that the eyepoint of most oculars is so close to the eyelens that one cannot get the eye close enough to the ocular if spectacles are worn. This is especially true if the curved toric glasses are used.

To make it possible to wear spectacles for microscopic observation, Swift of London has produced an ocular — " telaugic " (αυγή, eye; τῆλε, far) — of two combinations having a high eyepoint. With such an ocular the spectacles can be worn without interfering in any way, and with the advantage of the correction to eye defects which the glasses give. Twenty-six (26) oculars of all types and of five different makers were examined for the distance of the eyepoint from the eyelens of the ocular, that is, the height of the eyepoint. For measuring the height of the eyepoint a Beck swing-out lens holder (fig. 57) was used. In place of the magnifier, a piece of ground glass less than 1 mm. thick was put on the holder. The microscope was focused on a very transparent object and brilliantly lighted with a daylight lamp (fig. 199).

With the ground glass over the ocular it was very easy to find and to focus the eyepoint. The distance from the upper surface of the eyelens to the ground glass was then measured with a millimeter scale. To make sure that the eyepoint was sharply focused and that the measurement of the height was accurate, a low-power magnifier was used. It was found that the eyepoint was practically constant in height with objectives from 20 (8x) to 2 (90x) mm. equivalent focus, consequently the low power was used throughout.

The following is a summary of the findings:

5 different 5x oculars Height of eyepoint	10–14 mm.
6 different oculars, 6x, 6.45x, 7x and 7.5x	7–10 mm.
9 different oculars, 10x	6–20 mm.
4 different oculars, 15x	4 5–18 mm.
1 Compensation ocular of 20x	12 5 mm.
1 Periplane ocular of 25x	2 00 mm.
1 Telaugic ocular of 10x	20 00 mm.

From this summary it will be seen that there is at present great variety in height of eyepoint even for the same magnification, therefore it seems quite possible to construct a series of oculars for users of spectacles. See " telaugic " oculars, § 41.

§ **146. Centering of the ocular.** — From the size of the lenses of oculars, an exact correspondence of the optic axis of the ocular with that of the objective and the condenser is not so essential as with the condenser and objective. The tubes of microscopes are so mechanically perfect that when the ocular is in place its axis will be sufficiently near the center.

§ **147. Changing objectives and centering.** — With student microscopes and those for most purposes are now so well made that one need not worry overmuch about the centering in passing from one objective to another by means of a revolving nose-piece. If one wishes to test a microscope for centering of the condenser with the different objectives the directions given in § 118 will serve as a guide. Of course it is very easy for any one to see whether an object in the center of the field with one objective is also in the center of the field when the other objectives are swung into position.

§ **148. Exact centering impracticable.** — A perfect microscope would have the condenser centered to each objective, and at the same time an object in the center of the field with one objective would be in the center for all the others on the revolving nose-piece or with any other form of objective changer. While the approximation is good, no microscope has yet been tested which shows the perfect centering of both condenser and field; and microscopes of the principal makers have been critically examined for this information.

On the whole the revolving nose-pieces have been found as accurate as the more expensive objective changers; and the revolving nose-piece is very much more convenient.

In exacting research and with the dark-field microscope the question of centering is a very vital one. For research, the condenser should not only be of the best quality (§ 128), but it should be supplied with a centering device (fig. 60) by which its axis may be put in line with the axis of the objective being used. From rigid tests with many microscopes of many different makes the writer is forced to the conclusion that if successive objectives are to be centered, none of the objective changers or revolving nose-pieces are sufficiently accurate. The different objectives must be screwed directly into the lower end of the body tube of the microscope. Even then there will be slight differences, for so exacting are the requirements that no two objectives have yet been found by me that put in place successively will be exactly centered. The difference is usually very slight, much less than with a revolving nose-piece, but still the centering is not perfect. For the most perfect correlation the substage condenser should be centered for each objective.

ADJUSTABLE, WATER AND HOMOGENEOUS IMMERSION OBJECTIVES

Experiments

§ **149. Adjustment for objectives.** — As stated above (§ 29) the aberration produced by the cover-glass (fig. 64) is compensated for by giving the combinations in the objective a different relative position than they would have if the objective were to be used on uncovered objects. Although this relative position cannot be changed in unadjustable objectives, one can secure the best results of which the objective is capable by selecting covers of the thickness for which the objective was corrected. Adjustment may be made also by *increasing* the tube-length for covers *thinner* than the standard and by *shortening* the tube-length for covers *thicker* than the standard.

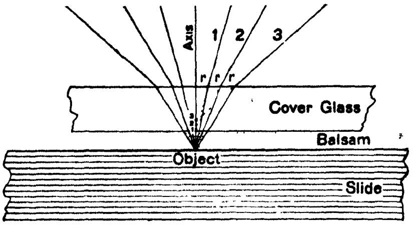


FIG. 64. ABERRATION PRODUCED BY THE COVER-GLASS.

Axis The extension of the principal optic axis.
Cover The cover-glass.
1, 2, 3 Three rays originating from the object mounted in balsam.
r, r, r Points of refraction as the three rays emerge from the upper surface of the cover into the air.
O Object from which the rays originate.
1, 2, 3 The three levels from which the rays seem to originate when traced backward from their points of emergence. This gives the effect of spherical aberration (Ch. IV).

In learning to adjust objectives, it is best for the student to choose some object, like Pleurosigma whose structure is well agreed upon, and then to practise lighting it, shading the stage and adjusting the objective, until the proper appearance is obtained. The adjustment is made by turning a ring or collar which acts on a screw and increases or diminishes the distance between the systems of lenses, usually the front and the back systems (fig. 44).

§ 150. Directions for adjustment. — (1) The thicker the cover-glass, the closer together are the systems brought by turning the adjusting collar from the zero mark and conversely; (2) the thinner the cover-glass, the further must the systems be separated, i.e., the adjusting collar is turned nearer the zero or the mark "uncovered." This also increases the magnification of the objective (§ 368).

The following specific directions for making the cover-glass adjustment are given by Mr. Wenham (Carpenter, 7th Ed., p. 166): "Select any dark speck or opaque portion of the object, and bring the outline into perfect focus; then lay the finger on the milled-head of the fine motion and move it briskly backwards and forwards in both directions from the first position. Observe the expansion of the dark outline of the object, both when within and when without the focus. If the greater expansion or coma is when the object is without the focus, or farthest from the objective [i.e., in focusing up], the lenses must be placed further asunder, or toward the mark uncovered [the adjusting collar is turned toward the zero mark, as the cover-glass is too thin for the present adjustment]. If the greater expansion is when the object is within the focus, or nearest the objective [i.e., in focusing down], the lenses must be brought closer together, or toward the mark covered [i.e., the adjusting collar should be turned away from the zero mark, the cover-glass being too thick for the present adjustment]." In most objectives the collar is graduated arbitrarily, the zero (0) mark representing the position for uncovered objects. Other objectives have the collar graduated to correspond to the various thickness of cover-glasses for which the objective may be adjusted. This seems to be an admirable plan; then if one knows the thickness of the cover-glass on the preparation (§ 518) the adjusting collar may be set at a corresponding mark, and one will feel confident that the adjustment will be approximately correct. It is then only necessary for the observer to make the slight adjustment to compensate for the mounting medium or any variation from the standard length of the tube of the microscope. In adjusting for variations of the length of the tube from the standard it should be remembered that: (1) If the tube of the microscope is longer than the standard for which the objective was corrected, the effect is approximately the same as thickening the cover-glass, and therefore the systems of the objective must be brought closer together, i.e., the adjusting collar must be turned away from the zero mark. (2) If the tube is shorter than the standard for which the objective is corrected, the effect is approximately the same as diminishing the thickness of the cover-glass, and the systems must therefore be separated (fig. 44), i.e., turned toward the zero mark.

In using the tube-length for cover correction shorten the tube for too thick covers, and lengthen the tube for too thin covers.

Furthermore, whatever the interpretation by different opticians of what should be included in tube-length, and the exact length in millimeters, its importance is very great, for each objective gives the most perfect image of which it is capable with the tube-length for which it is corrected, and the more perfect the objective the greater the ill effects on the image of varying the tube-length from the standard. The plan of designating exactly what is meant by tube-length and engraving on each objective the tube-length for which it is corrected, is to be commended, for it is manifestly difficult for each worker with the microscope to find out for himself for what tube-length each of his objectives was corrected (see Ch. IV).

§ **151. Water immersion objectives.** — Put a water immersion objective in position (§ 84) and the fly's wing for object under the microscope. Place a drop of distilled water on the cover-glass, and with the coarse adjustment lower the tube till the objective dips into the water, then light the field well and turn the fine adjustment one way and another till the image is clear. Water immersions are exceedingly convenient in studying the circulation of the blood, and for many other purposes where aqueous liquids are likely to get on the cover-glass. If the objective is adjustable, follow the directions given in § 150.

When one is through using a water immersion objective, remove it from the microscope and with some lens paper wipe all the water from the front lens. Unless this is done dust collects and sooner or later the front lens will be clouded. It is better to use distilled water to avoid the gritty substances that are likely to be present in natural water, as these gritty particles might scratch the front lens.

REFRACTION AND COLOR IMAGES

§ **152. Refraction images are those mostly seen in studying microscopic objects.** — They are the appearances produced by the refraction of the light on entering and on leaving an object. They therefore depend (*a*) upon the form of the object, (*b*) upon the relative refractive powers of object and mounting medium. With such images the diaphragm should not be too large (§§ 121–122).

If the color and refractive index of the object were exactly like the mounting medium, it could not be seen. In most cases both refractive index and color differ somewhat; there is then a combination of color and refraction images which is a great advantage. This combination is generally taken advantage of in histology. The air bubble in § 333 is an example of a purely refractive image.

A purely refractive image like that given by an air bubble or a fat globule gives a dark border for central transmitted light, and a light border on a black field with very oblique light, such as is given by the mirror turned far to one side or by a central stop when the condenser is used (§§ 138, 340). In both cases the object is in outline. As pointed out by Wright (p. 5, 41) the visibility of the object shown in outline depends on the width of the outline and not on the diameter of the whole object. If the width of the outline is too narrow to include the necessary visual angle of 1 minute (§ 360) the whole object fades into the background and is no longer visible. On the other hand, if the object is colored, then it is visible so long as its entire diameter gives a visual angle of 1 minute or more.

One can see from the above what a tremendous advantage it is in studying the finest details of structure to have them brilliantly colored.

HOMOGENEOUS IMMERSION OBJECTIVES

Experiments

As stated above (§ 23), these are objectives (fig. 44I) in which a liquid of the same refractive index as the front lens of the objective is placed between the front lens and the cover-glass.

§ **153. Refraction images.** — Put a homogeneous immersion objective in position; employ a condenser. Use some histological specimen like a muscular fiber as object; make the diaphragm opening about 9 mm. in diameter, add a drop of the homogeneous immersion liquid, and focus as directed in § 89. The object will be clearly seen in all its details by the unequal refraction of the light traversing it. The difference in color between it and the surrounding medium will also increase the sharpness of the outline. If an air bubble preparation (§ 334) were used, one would get pure refraction images.

§ 154. Color images. — Use some stained bacteria as Bacillus tuberculosis for object. Put a drop of the immersion liquid on the cover-glass or on the front lens of the homogeneous objective. Remove the diaphragms from the illuminator or in case the iris diaphragm is used, open it to its greatest extent. Focus the objective down so that the immersion fluid is in contact with both the front lens and the cover-glass; then with the fine adjustment get the bacteria in focus. They will stand out as clearly defined colored objects on a bright field.

If one closes the diaphragm until one-half or three-quarters of the aperture of the objective is used, the image will be a combined color and refraction image.

§ 155. Shading the stage of the microscope and the eyes of the observer. — As emphasized before, the clearest possible image of an object can be obtained when the only light reaching the eye comes from the object. With opaque objects and with the dark-field microscope this is literally true. With the bright-field microscope where the light is transmitted through and around the object it is necessary to exclude any other light than that which is transmitted by shading the stage of the microscope so that the image will not be blurred by light upon the object from various angles above the stage. This shading is easily accomplished by means of a screen (fig. 42) if daylight is used, or by having a shield as shown with the chalet microscope lamp (fig. 46). Both the screen and the shield in the daylight lamp also keep the light from the eyes of the observer.

§ 156. Removing homogeneous immersion liquid from glass surfaces. — Homogeneous immersion objectives, condensers and other glass surfaces covered with cedar oil or other homogeneous liquid are cleaned as follows: — The main part of the liquid is removed by a clean piece of gauze, then a piece of gauze or lens paper is wet with xylene or chloroform and the glass surface wiped. Immediately afterward a fresh piece of the lens paper is used to wipe away the last traces of the solvent. This leaves the glass surfaces clean and ready for the next experiment.

EXPERIMENTS WITH BINOCULAR MICROSCOPES

§ **157. Arranging the microscope for binocular vision.** — Until one has had some experience with binocular microscopes it is not easy to tell whether one is seeing with one eye or with both. In order to see with both eyes it is necessary that each eye should receive the beam of light from its own ocular at the same time, and this can occur only when the oculars are spread the right amount to bring the eyepoints the same distance apart as the pupils of the eyes of the observer, and the eyes are at the correct level.

Hold the head close to the oculars and look into the microscope. Focus as usual and the image will be satisfactory. Now to tell whether the image is seen with one eye or with both, hold the head still and shut the eyes alternately. If only one eye is being used no image at all will be seen when that eye is closed, but when the other is closed there will be no change in the appearance (§ 158).

If it is found that only one eye is being used, change the spread of the oculars by grasping the prism holder or drums or the tubes above these with the two hands and increase and diminish the distance between the tubes until both eyes are receiving the light, and there is an image in each eye. When this occurs and one once gets the stereoscopic effect there will never be any doubt in the future whether the vision is monocular or binocular.

§ **158.** — In some makes of binocular microscopes (the Spencer Lens Co.'s, for example), there is a little shutter just above the objectives which can be turned to either side, covering the back of the corresponding objective. If the image is still apparent whichever objective is covered then, of course, both eyes are seeing the image, but if the image is wholly obliterated when the shutter is on one side, that is the only side giving an image, and the tubes must be changed in position to get the correct pupillary distance of the eyepoints.

EXPERIMENTS WITH DOUBLE-OBJECTIVE BINOCULAR MICROSCOPES

§ **159. Opaque and transparent objects.** — Place the binocular microscope (fig. 27) near a window where there is an abundance of light or if artificial light must be used, employ the dark-field lamp (figs. 79 or 80) or a bull's-eye condenser (fig. 127) to concentrate the light upon the object. At first use low power objectives and oculars. As it is somewhat easier to get the stereoscopic effect with opaque objects, use a black background like a piece of black velvet. Put a flower or some folded white gauze, a bunch of keys or other familiar object under the microscope and look at the object. Focus sharply. Make sure that both eyes see the image as directed above (§§ 157, 158).

After trying various opaque objects, and becoming familiar with the necessary adjustments, use a large transparent object like a preparation with the blood vessels injected. The different levels of the blood vessels will stand out with amazing distinctness.

The double-objective binoculars are excellent for studying the circulation of the blood and all injected preparations. For dissection the microscope is mounted on an arm which may be swung into position.

§ **160. There are three precautions to keep in mind:** The oculars must be the right distance apart for the observer's pupillary separation; (2) the two oculars must be of the same power; and (3) finally the observer must make sure that the image is in focus for both eyes. In all the best modern binoculars of all kinds adjustments are provided for this purpose.

If the special focusing device for eye differences is at the left as in fig. 27, then one closes the left eye and focuses the microscope for the right eye as sharply as possible. The right eye is then closed and the image examined with the left eye. If it is equally sharp with the left eye, the microscope is properly adjusted for both eyes, and will give a good binocular image. If the image should not be sharp for the left eye, then without changing the focus of the microscope, one turns up and down with the focusing device on the left objective, until the image is sharp to the left eye. Make sure that it is also sharp for the right eye. If it should not be, one must repeat the entire operation. In this way one can have a perfect image in each eye.

Correct movement of the specimen or instruments under an erecting microscope. — For one who has become thoroughly trained in using the ordinary inverting compound microscope it is very difficult to make the proper motions to move the specimen, or to move the dissecting instruments correctly under an erecting compound microscope. This illustrates the power of training. The beginner with the inverting microscope finds it hard to move his hands in the opposite way from what his eyes dictate, but when the correlation between the appearance and the motion necessary has become fixed, it is equally difficult to move the hands in the direction which the eyes indicate, although it is known that this is now correct. This difficulty is soon overcome by practice.

Under the simple microscope, however, in which there is no reversal or inversion, the eyes and hand work together automatically as with the naked eye.

EXPERIMENTS WITH SINGLE OBJECTIVE BINOCULARS

§ **161. Experiments with low powers.** — Arrange the binocular microscope so that it stands squarely before you, otherwise it will not be easy to hold the head so that the eyes are directly over the eyepoints of the two oculars.

As it is simpler, use first an opaque object like some loosely woven gauze or other white cloth, a light colored insect or other opaque object with very definite features which are at different levels. Light well by having the microscope before the window or by the use of a bull's-eye lens or best of all by the use of the dark-field microscope lamp. Use a low objective, one not higher than 16 mm. (10x) and low oculars x5 or x6, and make sure that the oculars are of the same power. With the eyes in the correct position and the object well lighted there should be no difficulty in getting the stereoscopic effect. It is well also to close one eye and get the appearance, and then the other, or to use a monocular microscope and compare the appearance of the object with monocular and with binocular vision. For many who have had considerable experience, the image looks just as stereoscopic with one eye as with both.

For transmitted light, use some translucent object like a section in which the blood vessels have been injected. These are thick and when well lighted by the mirror or the mirror and condenser show the stereoscopic effect very strikingly. Try all powers, including the homogeneous immersion. A fly's wing mounted in balsam is good for all powers.

§ **162. Unlikeness of the two eyes.** — If the two eyes are markedly unlike, true binocular vision is impossible. If the difference is not great, correction can be made with spectacles, or with the special focusing adjustment on one side.

With the mon-objective binoculars the correcting device for unlikeness of focus of the two eyes is usually on the left tube below the ocular. It may be on the right as in fig. 35. To make sure that the two eyes have sharp images, proceed as described for the double-objective binocular (§ 160), only in this case the correcting device is on the tube of the microscope and not on the objective.

§ 163. Experiment with unlike oculars. — It occasionally happens that oculars of different powers get into the two tubes of the binocular. It produces great confusion as one can see by an experiment. Use some well known object with *like oculars* and get the image as perfect as possible, then put a higher or lower ocular in one of the tubes. Get both images sharp as directed in § 162. If both eyes are then opened there will not be a good single image of the two differently magnified images, although separately both are good.

§ 164. Change from binocular to monocular observation. — The method first adopted was the removal of the binocular arrangement and the substitution of a monocular tube. It takes only a moment to make the change (fig. 32). The latest device is to slide the binocular arrangement sidewise. When this is done the prism of one tube is swung aside and this gives one tube in the main axis of the microscope to serve in place of the single tube formerly employed.

TESTING THE MICROSCOPE

§ 165. Testing the microscope. — To be of real value this must be accomplished by a person with both theoretical and practical knowledge, and also with an unprejudiced mind. Such persons are not common, and when found do not show overanxiety to pass judgment. From the writer's experience it seems safe to say that the inexperienced can do no better than to state clearly what he wishes to do with a microscope and then trust to the judgment of one of the optical companies. The makers of microscopes and objectives guard with jealous care the excellence of both the mechanical and optical part of their work, and send out only instruments that have been carefully tested and found to conform to the standard. This would be done as a matter of business prudence on their part, but it is believed by the writer that microscope makers are artists first and take an artist's pride in their work; they therefore have a stimulus to excellence greater than business prudence alone could give.

What has just been said does not by any means imply that the purchaser of a microscope should blindly accept anything which is offered him. It simply means that if one has no knowledge of a microscope one can hardly pass expert judgment upon it.

§ 166. **Mechanical parts.** — All of the parts should be firm, and not too easily shaken. Bearings should work smoothly. The mirror should remain in any position in which it is placed (fig. 26).

§ 167. **Focusing adjustments.** — The coarse or rapid adjustment should be by rack and pinion and work so smoothly that even the highest power can be easily focused with it by an experienced observer.

This coarse adjustment is liable to work too hard or too easily. If it works too hard, the bearings of the pinion are too tight or the gliding surfaces are sticky and not properly lubricated. If the bearings are too tight, loosen the screws very slightly; if the bearings are not lubricated or the surfaces are covered with sticky oil, wet a cloth with a good lubricating oil and rub the gliding surfaces well. This will clean them and lubricate them at the same time.

If the tube runs down too easily the bearings of the pinion are too loose and the screws should be tightened a little.

§ 168. **The fine adjustment is more difficult to deal with.** — From the nature of its purpose, unless it is approximately perfect, it would be better off the microscope entirely. It has been much improved recently.

It should work smoothly and be so balanced that one cannot tell by the feeling when using it whether the screw is going up or down. Then there should be absolutely no motion except in the direction of the optic axis; otherwise the image will appear to sway even with central light. Compare the appearance when using the coarse and when using the fine adjustment. There should be no swaying of the image with either if the light is central (§§ 133-134).

§ 169. Testing the optical parts. — As stated in the beginning, this can be done satisfactorily only by an expert judge. It would be of very great advantage to the student if he could have the help of such a person. In no case is a microscope to be condemned by an inexperienced person. If the beginner will bear in mind that his failures are due mostly to his own lack of knowledge and lack of skill, and will truly endeavor to learn and apply the principles laid down in this and in the standard works referred to, he will learn after a while to estimate at their true value all the parts of his microscope.

If one can compare a new or unfamiliar microscope with one with which there is entire familiarity, a very good estimate can be made. *The first principle is to use some microscope with which one is familiar and to use microscopic preparations of which one knows the structure;* then a fair judgment can be made of the excellence of the performance of the new instrument. If there seems to be any defect in the image, make sure

(1) that the lighting is good;

(2) that the proper aperture of the objective is being used and that the condenser is centered; (§§ 123, 118).

(3) that the stage is shaded;

(4) that the tube-length of the microscope is that for which the objectives were corrected.

(5) that the preparation is clean and gives a good image with the microscope with which one is familiar. If all the precautions have been taken and still a good image cannot be obtained one should get some more expert friend or the makers to show wherein the trouble lies.

COLLATERAL READING

The same as for Chapter I. Consult the catalogues of the Microscope Manufacturers, and the small guides they send out with their microscopes.

CHAPTER III

THE DARK-FIELD MICROSCOPE AND ITS APPLICATION

§ 170. Comparison of bright-field and dark-field microscopy. — In most work with the microscope the entire field of view is lighted and the objects to be studied appear as colored pictures or as shadows — in extreme cases, as silhouettes — on a white ground. As the field is always light, this has come to be known as Bright-Field Microscopy.

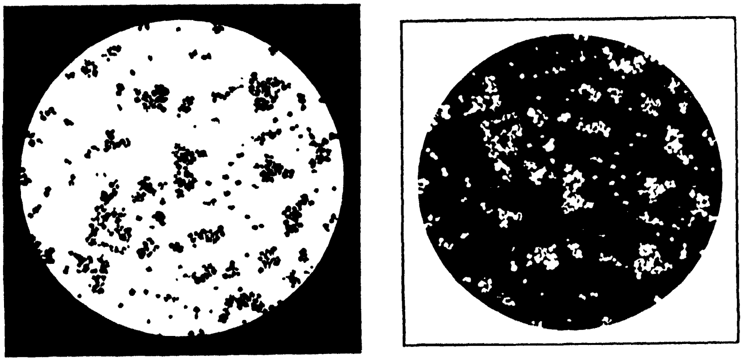


FIG. 65. FIG. 66.

Bright- and dark-field photo-micrographs of the same objects (starch grains).

In contrast with this is Dark-Field Microscopy in which the field is dark, and the objects appear as if they themselves emitted the light by which they are seen.

The study of objects in a bright-field probably comprises 95 per cent of all microscopic work, and is almost universally applicable. On the other hand dark-field microscopy has a more limited applicability, and yet from the increased visibility given to many objects it is coming to be appreciated more and more.

§ **171. Definition of dark-field microscopy.** — In its comprehensive sense, Dark-Field Microscopy is the study of objects by the light which the objects themselves turn into the microscope.

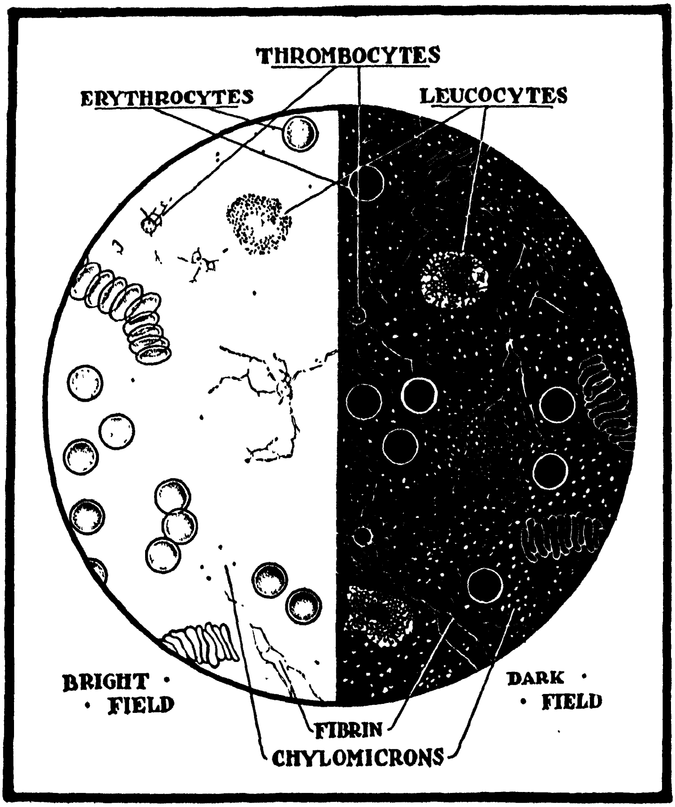


FIG. 67. FRESH BLOOD.

Half the field is with dark-ground and half with bright-ground illumination.

There are two principal cases: (A) The objects which are truly self-luminous like phosphorescent animals and plants; burning or incandescent objects, and fluorescent objects. (B) The objects which emit no light themselves, but which deflect the light reaching them from some outside source into the microscope.

These two groups are well represented in astronomy. If one looks into the sky on a cloudless night, the fixed stars show by the light which they themselves emit, but the moon and the planets appear by the light from the sun which they reflect to the earth, the sun itself being wholly invisible at the time. As there is relatively very little light coming from the intervening space between the stars and planets, all appear to be self-luminous objects in a dark field. This reference to the sky at night will serve to bring out two other points with great clearness: (1) The enhanced visibility. Everybody knows that there are as many stars in the sky in the daytime as at night, but they are blotted out, so to speak, by the flood of direct light from the sun in the daytime, while at night when these direct rays are absent and no light comes from the background the stars and the planets show again by the relatively feeble light which they send to the earth.

(2) The other point is that in dark-field microscopy the objects must be scattered, not covering the whole field (fig. 66).

§ **172. Light in the workroom.** — As brought out in the previous section in referring to the stars, they appear brighter in a dark clear night, so with dark-field microscopy. If one works at night or in a dark room the effects are more satisfactory for two reasons: (1) The scattered light of daylight or lamp light does not enter the eye and thus lessen the effect of the dark-field appearances in the microscope.

(2) In a darkened room the eyes of the observer are adapted for relatively dim light and therefore the details of structure in the dark-field microscope are apparently emphasized, just as when one goes into a relatively dark room from a brightly lighted one. At first almost nothing can be seen, but when the eyes become adapted to the dim light, much can be seen.

§ **173. Dark-field and ultra-microscopy.** — In both of these the objects seem to be self-luminous in a dark-field, and no light reaches the eye directly from an outside source, but only as sent to the eye from the objects under observation.

The terms simply represent two steps, and merge into each other.

Dark-Field Microscopy deals mainly with relatively large objects, 0.2μ or more in diameter, that is, those which come within the resolving power of the microscope.

Ultra-Microscopy deals principally with objects so small that they do not show as objects with details, but one infers their presence by the points of light which they turn into the microscope. This can be made clear by an easily tried naked-eye observation. Suppose one is in a dark room, and a minute beam of brilliant light like sunlight or arc light is directed into the room. Unless one is in the path of this beam of light, it will remain invisible; but if there are vapor or dust particles present, they will deflect some of the light toward the eye and will appear as shining points. The character of the particles cannot be made out, but the points of light they reflect indicate their presence. As Tyndall used this method in determining whether a room was free from dust in his experiments in spontaneous generation, the appearance of the shining dust particles is sometimes called the "Tyndall effect."

The two forms are said to merge, because in studying objects like saliva, etc., with the microscope designed especially for dark-field work, some of the objects seen will show details, but some are so small that they show simply as points of light usually in the form of so-called diffraction discs. The larger objects in the saliva come in the province of dark-field microscopy, and the smallest ones, of ultra-microscopy, and in this case the instrument used might with equal propriety be called a dark-field or an ultra-microscope.

§ 174. Visibility and resolution with the dark-field microscope. — Visibility refers only to the possibility of seeing that some object is present; resolution to the possibility of seeing details so that one can judge not only that an object is present but also see some of the structure and relations of the object. These two terms come over from the ancient science of astronomy where the questions were whether what seemed a single bright point in the sky was a single star or a double or triple star or a star cluster; and whether the surface of the planet Mars was uniform or had markings and whether the planet Venus was always a round disc of light or had phases like the moon. The telescope, which increased the visual angle under which the different things could be seen, added a certain amount of resolution, and details were made visible which are invisible to the naked eye.

It is contended by some workers that the whole purpose of the dark-field microscope is to make objects visible, just as the stars and planets are visible on a clear, dark night. Other persons are equally emphatic that the dark-field microscope not only makes objects visible, but it also is a powerful aid in resolution, bringing out details of structure not even visible with the bright-field microscope. The matter is admirably stated by Beck, and has the writer's emphatic endorsement: With the dark-field microscope when skillfully used, "There is no glare or flooding and the whole aperture of the object-glass is evenly filled with light so as to give the maximum resolution. There is no foundation for the statement that has been made that this form of illumination does not give the full resolving power of the object-glass in use. Anything that can be resolved by transmitted illumination can be resolved by dark-ground illumination, and in general with much greater brilliancy, because of the increased contrast between different parts of the structure."

§ 175. Naked-eye demonstration of dark-field effects. — Use some black velvet and scatter upon it some minute pieces of white paper, also a small piece of black velvet. Place in a well lighted window or light well by a bull's-eye (fig. 68) or the dark-field lamp (fig. 80).

The paper reflects the light to the eyes; the small piece of velvet is very obscure. For comparison with bright-field appearances, use a piece of white paper and put on it small pieces of white paper and a piece of the black velvet. These experiments bring out clearly the advantages of contrast as well as the light and dark background.

§ 176. Dark-field effects with light above the stage. — Use a 16 mm. (10x) or lower objective, and a low ocular. Place some black velvet on the stage of the microscope, and upon it a glass slip on which are some grains of flour or starch or other white powder. Place the microscope near a well lighted window or let the light from a lamp fall upon the top of the slip (fig. 68). In the microscope the particles will appear brilliantly white on a dark background. In all work with the microscope, whether with bright- or dark-field illumination, one would do well to remember this experiment, for it illustrates how light from above the stage may enter the microscope. Such scattered, irregular light often spoils the sharpness of the image with transmitted light. This is why it is best to have no light strike the object from above the stage when the lighting is from below the stage, whether for bright- or for dark-field illumination.

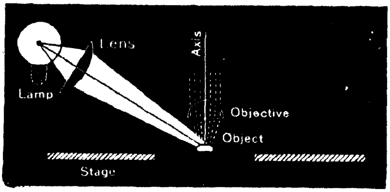


FIG. 68. LAMP WITH PLANO-CONVEX LENS ABOVE THE STAGE.

§ 177. **Dark-field effects with light from below the stage.** — As commonly understood, dark-field illumination refers especially to light from below the stage. The light may be directed upon the object from some source as in fig. 68, or it may be reflected from a mirror. For the higher powers it is necessary also to use a condenser to light the object with a sufficient aperture.

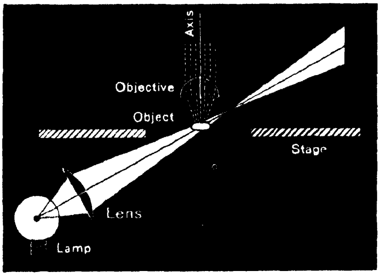


FIG. 69. LAMP AND BULL'S-EYE LENS BELOW THE STAGE.

Use the same microscope and the same specimen as in § 176. Swing the mirror far to one side and direct the light very obliquely upon the glass slip supporting the white granules; or use a bull's-eye to direct the light very obliquely upon the object. The purpose is to have the light all so oblique that none of it can get directly into the objective. The only light that should pass into the objective is that reflected from the white granules. If light were to pass directly into the objective the background would be light.

Dark-Field Illumination by the Aid of Condensers

Just as with the bright-field microscope the mirror alone does not give light of high enough aperture for the modern objectives used and a condenser must be employed, so with the dark-field microscope a condenser must be used to give rays of sufficient obliquity. With the bright-field microscope the light illuminating the object is in the form of a solid cone (figs. 61, 287–290), but with the dark-field microscope, where only oblique rays must light the object, the central part of the solid cone must be blocked out somewhere in its course and only the oblique rays forming a hollow cone of light must be permitted to illuminate the object. If the objects in the field are thus illuminated by rays so oblique that none of them can enter the objective directly, the objects will appear bright by the light which they deflect into the microscope and the background or field will be dark; that is, the objects will appear to be self-luminous in a black field, as with the moon and the planets in the sky at night.

The light reaching the condenser from the mirror is mostly in the form of a solid beam of parallel rays, and the stop to eliminate the central part of the beam may be below all the elements of the condenser as in figures 70, 84, 279, or it may be between the elements of the condenser as in figures 71, 77, 280–282. In all cases the central part is blocked out and only the outer shell or hollow cone composed of the most oblique rays is permitted to pass on and illuminate the object. This is shown in the illustrations of all the dark-field condensers (figs. 71, 74, 77, 84, 126, 279, 280–281) except figure 70.

§ **178. Spot lens and refracting condensers for dark-field effects.** — Besides the early devices for dark-field shown in figures 68–69, a condensing lens with a black central patch cemented to the middle was much used. Since 1854 at least, the achromatic and non-achromatic bright-field condensers have been found to give excellent black backgrounds if central stops are used. (See Ch. XIV.)

In general, these refracting condensers are entirely satisfactory for use with low objectives, that is, those of 8 (20x) to 16 mm. (10x) equivalent focus and still lower ones. (See also §§ 717–723.) They have the further advantage of lighting a large field. By using care in lighting, by selection of slips of the correct thickness and by the use of brilliant light, dark-field work can be done satisfactorily with objectives of 8 mm. (20x) and 4 mm. (e.f.) (40x), provided the numerical aperture does not exceed 0.60 to 0.66. Homogeneous immersion objectives of 2 (90x) to 3 mm. (e.f.) (60x) and indeed dry objectives of any power and aperture can be used provided there is inserted a reducing diaphragm to bring the aperture slightly below 0.65. For the higher powers one must use a strong light and it is also of great advantage to use homogeneous immersion liquid between the condenser and glass slip bearing the object, for then the most oblique rays can pass into the slip and illuminate the object up to the refractive index of its mounting medium. For the 16 mm. (10x) and lower objectives immersion contact of the condenser and slip is unnecessary unless especial brilliancy is needed.

§ 179. Thickness of slips to use with refracting condensers. — The object should be in the focus of the condenser (figs. 70, 77), hence the slip used should be of the thickness to put the objects upon it at the level of the focus. This is, of course, more important with the higher powers than with the lower ones. The ordinary Abbe condenser does not have a sharp focus on account of its aberrations, consequently one need not be so particular about selecting the slips. With the achromatic condensers, however, there is a sharp focus and, as a rule, it is quite near the surface of the top of the condenser, hence thin slips should be used. Fortunately many microscope makers give the equivalent focus of their condensers and also the working distance, that is, the distance between the top of the condenser and its focus. This working distance shows the thickness of slip to use, and if the slip is of that thickness, it will bring the focus of the condenser at the upper surface of the slip where the object is situated, and thus insure the most brilliant illumination.

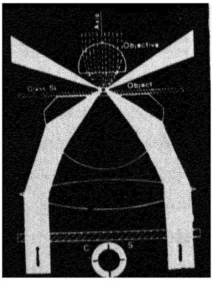


Fig. 70. Refracting Condenser with Central Stop (C–S) for Dark-Field Illumination.

If the makers do not state the equivalent focus and working distance, one can find it by holding the condenser with its lower end toward the sun. The sun's rays are practically parallel and hence will be brought to a focus above the upper end of the condenser. If one measures the distance between the focal point and the top of the condenser it will show the working distance. A piece of ground glass is a good object to use to show where the focal point of the condenser is situated. This working distance indicates the thickness of the slide to use.

§ **180. Size of the central stop required.** — Evidently the central stop must be of a size to exclude all rays which could pass directly into the microscope objective, and allow those to pass which were of an aperture greater than that of the objective in use. The size can be easily determined in any given case as follows: The iris diaphragm is opened widely, and the light reflected up through the condenser. The objective should be focused on a transparent specimen; the ocular is removed and one looks directly down the tube of the microscope. The back lens of the objective will be brilliantly lighted. Now slowly close the iris, and soon its edge will be seen all around the bright area. Close the iris until sure that it is clearly seen. Then slowly open it until its opening is just at the edge of the back lens of the objective. The use of the eyepoint magnifier or the pinhole cap is convenient in this connection (figs. 57-58). This opening of the iris which just fills the aperture of the objective indicates how large a central stop is necessary to exclude all this light. Turn the microscope over on its side and, with fine pointed dividers, measure the diameter of the iris opening. Make a central stop of the size of the iris opening or slightly larger out of thick paper like a visiting card. As shown in figure 70 there must be three or four arms left to support the central stop. Blacken the paper with black ink, and put it in the holder under the condenser. If now a suitable object is put on a slip and the objective focused, there should be a dark-field, and the objects present should shine as if by their own light. If the field looks gray instead of black it is because the central stop is too small or is not centered, or the white particles used on the slip are far too numerous and do not leave enough blank space.

One can determine what is at fault thus: The ocular is removed. If the central stop is too small the back lens of the objective will show a ring of light around the outside. If the central stop is not centered there will be a meniscus of light on one side. If the objects are too numerous the whole field will be bright. To verify these statements one can use a specimen with flour or starch all over the slide.

For the meniscus of light when the central stop is decentered, purposely pull the ring holding the stop slightly to one side and the meniscus will appear in the back lens. To show the ring of light due to a too small size of the stop, the easiest way is to use a higher objective, say one of 3 (60x) or 4 mm. (40x), in place of the 16 mm. (10x) objective. While it is necessary to eliminate all the light which could enter the objective directly, the thicker the ring of light which remains to illuminate the objects, the more brilliantly self-luminous will they appear, therefore one uses only the stop necessary for a given objective. If one makes central stops for the different objectives as described above, it will be greatly emphasized that the objectives differ in aperture; in general the higher the power, the greater the aperture, and consequently the larger must be the central stop, and the thinner the ring of light left to illuminate the object. As one needs more light for high powers instead of less than for low powers, the deficiency of light caused by the large central stop must be made good by using a more brilliant source of light for the high powers.

§ 181. Dark-field element for refracting condensers. — Recently there has been devised a dark-field element for the improved Abbe condensers. The upper lens of the condenser is removed and in its place is a lens with the lower end ground away, and blackened directly or by means of a special diaphragm which can be placed very close to the lens. By this arrangement only the very oblique rays at the edge of the solid cone can enter the sides of the upper element, and dark-field illumination results as the rays in the hollow cone which illuminates the object are all too oblique to enter the objective directly (fig. 126).

These dark-field elements do not give so perfect dark-field illumi nation as do the regular paraboloid or reflecting condensers, but they do have the advantage that they are relatively cheap, and serve to light rather a large field. As with the regular dark-field condensers, it is better to have the slide in immersion contact with the condenser.

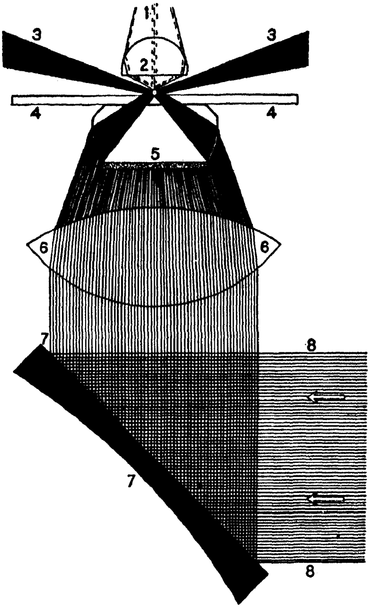


FIG. 71. REFRACTING CONDENSER WITH UPPER DARK-FIELD ELEMENT (5) IN PLACE.

(For full explanation, see fig. 126.)

§ 182. **Light for dark-field work with refracting condensers.** — For objectives of 8 mm. (20x), 16 mm. (10x), and lower powers, ordinary daylight or lamplight answers fairly well, and the Chalet microscope lamp answers for the 8 mm. (20x) objective. If the 4 mm. (40x) of 0.66 N.A., or the immersion objective reduced to 0.80 or 0.85 N.A. is to be used, then the dark-field microscope lamp gives more satisfactory results. For many objects it is advantageous to have a piece of finely ground glass in the path of the light. For all powers the light through the piece of daylight glass without any condenser (fig. 82) is good for many purposes. As for the reflecting condensers, it is well to keep in mind that too great intensity of light tends to make the background gray from the stray light which gets into the field.

§ **183. Objectives and oculars for dark-field work with refracting condensers.** — With the dark-field element in place one can use all powers, including an oil immersion, but the lower powers are the more satisfactory. For the 4 mm. and the oil immersion it is advantageous to have their iris diaphragms closed somewhat more than when using the cardioid or paraboloid condensers.

With the ordinary refracting condenser of 1.20 to 1.40 N.A., fairly good dark-field effects can be produced by using the proper dark-field stop (fig. 282) if the objectives of 8 mm. and higher powers have iris diaphragms that can reduce the apertures sufficiently. They light a large field and permit change from dark- to light-field without disturbing the specimen (§ 718).

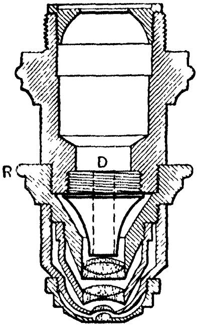


FIG. 72. HIGH-POWER OBJECTIVE WITH APERTURE REDUCING DIAPHRAGM FOR DARK-GROUND ILLUMINATION.
(From Chamot.)
D Funnel-shaped reducing diaphragm screwed into the lower end of the "boot" (opposite *R*).

REFLECTING DARK-FIELD CONDENSERS

As was first pointed out by Wenham, 1850–1856, refracting condensers are not so well adapted for obtaining the best ring of light for dark-field work as a reflecting condenser, on account of the difficulty in getting rid of the spherical and chromatic aberration in the refracted bundles of such great aperture. He first (1850) used a silvered paraboloid and later (1856) one of solid glass as is now used. Within the last 10 or 15 years there have also been worked out reflecting condensers on the cardioid principle (Fig. 77). The purpose of all forms is to give a ring of light which shall be of great aperture, and be as free as possible from chromatic and spherical aberration, and hence will form a sharp focus of the hollow cone upon the level where the objects are situated.

In the reflecting as in the refracting condensers the central part of the light beam from the source is blocked out by a central opaque stop and only a ring of light enters the condenser (figs. 70, 75, 77, 84).

While the purpose of the reflecting condenser is to produce a hollow cone of light of great aperture for illuminating the object, it is seen at once that the law of refraction will prevent the light from passing from the condenser to the object unless the glass slip bearing the object is in immersion contact with the top of the condenser.

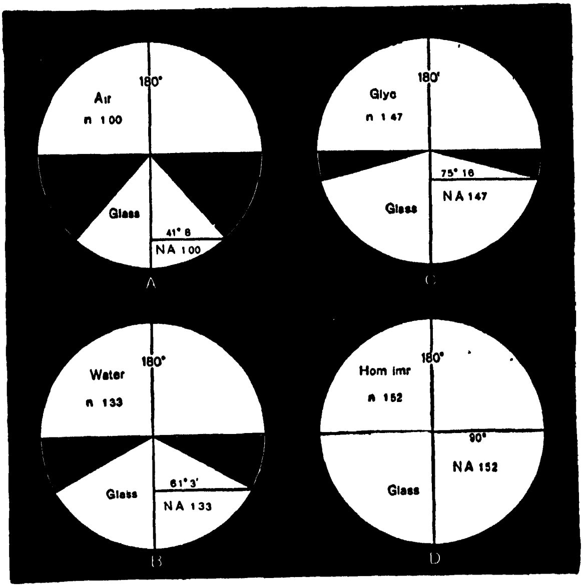


FIG. 73. HEMISPHERES OF GLASS TO SHOW THE REQUIRED ANGLE OF THE CONE OF LIGHT IN THE GLASS TO FILL THE OVERLYING HEMISPHERE WITH LIGHT.

The diagrams show that in each case the cone of light in the glass must have an aperture equal to the refractive index of the overlying medium: For air, *N A 1.00;* for water, *N A 1.33;* for glycerol, *N A 1.47;* for homogeneous liquid, *N A 1.52.* Any aperture of the light cone in the glass in excess of the refractive index of the medium above is beyond the critical angle and is therefore reflected back into the condenser.

If the objective is traversed by the light, then the aperture will be limited by the refractive index of the object. In like manner any medium between the objective and the object limits the aperture depending on the refractive index of the medium.

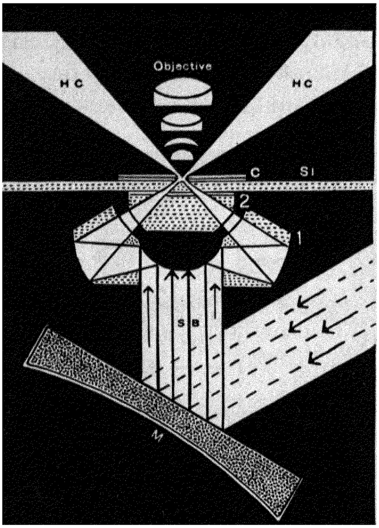


FIG. 74. BECK'S FOCUSING DARK-FIELD CONDENSER.

SB Solid beam of light from the mirror to the stop, which permits only the border rays to pass on to form the hollow cone of light.

1, *2* (*cd* in § 181) The lower movable and the upper fixed elements of the condenser. The closer the elements the thicker can be the slide, the farther separated the thinner.

Sl, *c* Slide and cover-glass in immersion contact below with the condenser and above with the objective.

Figure 73 shows the maximum aperture that can pass from the condenser to the object where there is (1) air, (2) water, (3) glycerin or (4) homogeneous immersion between the top of the condenser and the glass slip. If there is homogeneous contact then the only limit up to 1.52 N.A. is the mounting medium of the object itself.

§ 184. Numerical aperture of reflecting dark-field condensers. — This must be greater than the objective with which it is to be used, and the central part of the cone of light up to the full aperture of the objective must be stopped out, leaving a hollow cone of light all of whose rays are at a greater aperture than that of the objective.

By means of a screw the upper and the lower elements can be separated or approximated to change the position of the focus of the hollow cone and thus to make it possible to use slides of different thickness. The closer the elements the thicker can be the slide, the farther apart the elements the thinner must be the slide (Beck, p. 132).

In practice it has been found safer to reduce the aperture of the objective to 0.80 or 0.85 N.A., then one can get a dark-field with any good dark-field condenser. (The writer has not found the reducing diaphragms furnished by the makers to exceed 0.90, and some were even as low as 0.60 N.A.) See further under § 186.

§ 185. Homogeneous objectives for dark-field microscopy. — From the uncertainty in the use of these reducing diaphragms, it was urged in the former edition of this book that opticians prepare homogeneous objectives especially for dark-field work. As a result of tests of several of the standard reflecting dark-field condensers of both the paraboloid and the cardioid form, it was found that an objective aperture of 0.80 would give a dark-field, hence this aperture is mentioned as safe for all the standard makes, although in some cases an aperture as great as 0.90 could be safely used.

In 1921, at my earnest personal solicitation, the Bausch & Lomb Optical Company, and the Spencer Lens Company did construct homogeneous immersion objectives with an aperture of 0.80 to 0.85 N.A. for dark-field work. These have proved thoroughly satisfactory for the dark-field work and also for most of the work in histology and pathology where the substage condenser was used dry and therefore could not supply an aperture greater than 1.00 to the object and to the oil immersion objective. (See discussion of the need for a homogeneous immersion condenser, §§ 124–125.)

In England and on the Continent homogeneous immersion objectives of about 3 mm. (60x) focus have been produced for some time. Some of these have an aperture below 1.00 N.A. for dark-field work. In the special micro-catalogue of Zeiss No. 306, received in 1922, there was found an announcement of two apochromatic, homogeneous immersion objectives especially designed for dark-field work. They are designated X (60) (f. 3 mm.) and W (120) (f. 1.5 mm.). Each has an aperture of 0.85, and both are excellent for dark-field use and also for bright-field work.

§ 186. Dark-field condensers for high apertures. — During the last two years there have been developed in England by the veteran optician, Edward Nelson, and by the Messrs. Beck, dark-field condensers which permit the use of homogeneous immersion objectives up to 1.25 to 1.40 N.A. The type of Nelson's condenser is known as a Cassegrain reflecting condenser and is produced by Watson & Sons (fig. 75).

The high apertured condenser by Beck is known as a "Special Focusing Dark Ground Illuminator" and is said to give an illuminating aperture from 1.32 to 1.45 N.A. It is described in Beck's last catalogue and in Conrad Beck's book, "The Microscope," Part II, 1924, pp. 128–129. In general it is like fig. 74 which gives an aperture from 1.00 to 1.40. The special focusing condenser used with slips of 0.5 mm. thickness gives an aperture of 1.32 to 1.45, and objectives of an aperture of 1.25 can be used successfully with it.

While the Nelson Cassegrain reflecting condenser permits the use of high apertures, even up to 1.40 according to Dr. F. J. Brislee, President of the Liverpool Microscopical Society, in Watson's Microscopic Record, Sept. 1924, pp. 4–7, it must be remembered that such high apertures require a mounting medium of high refractive index, for the aperture of light finally entering the objective is limited by the medium of lowest refractive index between the objective and the object. It is also evident that objects like blood-corpuscles which are normally in a medium of about n D 1.33, cannot be viewed by an aperture greater than the mounting medium. Furthermore, the very great obliquity of the high-apertured light is a severe test of the corrections of the condenser, and is likely to cut down the intensity of the illumination.

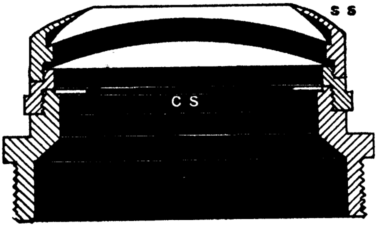


FIG. 75. NELSON'S CASSEGRAIN DARK-FIELD CONDENSER FOR IMMERSION OBJECTIVES OF APERTURE ABOVE *1.00 N A.*

(From Watson's Microscope Bulletin.)

c-s Central stop, these are of different size depending on the aperture of the objective to be used.

s-s Silvered border of the upper, reflecting element.

§ 187. Combined dark-field and bright-field condensers. — Every one who uses the dark-field microscope for serious work often feels the need of bright-field observation upon the identical object to make it possible to arrive at a just interpretation of a given appearance. To meet the insistent demand three Continental opticians have designed and manufactured three different types of combined condensers, and they all enable one to see the same structure by a slight change in the accessories of the condenser, but no change in the specimen under observation.

In the form of Leitz, by the change in position of a central stop and an iris diaphragm either bright- or dark-field illumination can be used, and when the iris is open and the central stop turned aside, both kinds of illumination can be used at the same time. This condenser is better for dark- than for bright-field illumination, and like the following, not so good for either as condensers especially designed for one purpose.

The form of Reichert is a superstage condenser in which is present, besides the upper element of the dark-field, a disk with central stops of different sizes, a ground glass for mild, low power bright-field lighting, and a very convex lens serving the purpose of an Abbe condenser. These different elements are brought into the optic axis by rotating the disk. This is a convenient and effective instrument, and when once centered, is satisfactory (§ 189).

The form of Zeiss is a modification of his paraboloid condenser by the addition of elements below the paraboloid proper and changed in position by means of two levers. One of the levers turns the central stop in position for dark-field work, and to one side for bright-field work. The other, shorter lever actuates an element which adjusts the condenser for slips of different thickness between 1.00 and 2.00 mm. This makes it possible to examine successfully preparations which were not especially mounted for dark-field work. On the whole this condenser is easiest to work with and gives satisfactory results with a wide variety of thickness of slip, and different magnifications.

All of these condensers require immersion contact of the slip and the top of the condenser. For low powers water immersion is sufficient. For high powers the homogeneous immersion contact is best, and it gives more brilliant pictures for all powers because of the additional aperture that it makes possible.

§ 188. Focusing dark-field condensers. — With dark-field condensers the thickness of the glass slip is determined by the fixed focal distance and working distance of the condenser, and unless the slip is of the corresponding thickness the object will be above or below the apex of the cone of light and therefore not in the most favorable position to bring out its details of structure (fig. 77).

Beck Limited of London and Zeiss of Jena have designed dark-field condensers in which the focal-point and consequently the working distance can be made to vary, and hence make possible the successful use of slips of different thickness. This is convenient when examining objects not originally mounted for dark-field work.

With the Beck form, by means of a lever below, the elements of the condenser (fig. 74c, d) can be separated or approximated for slips 0.5 to 1.85 mm. As evident from the diagram, the nearer *d* is to *c* the thicker the slip, and the farther *d* and *c* the thinner the slip. Furthermore with this form, by using a very thin slip (0.5 mm.), it is possible to increase the aperture of the condenser and hence to make it possible to use higher apertured homogeneous immersion objectives and thereby gain the advantage in resolution made possible by the added aperture. With the form figured, Beck says that with the adjustment made for a half millimeter slip and specimens mounted upon it, an aperture as high as 1.20 in the objective may be used. See also § 186. The Zeiss form is focused for different thickness of slips (1.00 to 2.00 mm.) by drawing a short lever to the right for thin and to the left for thick slips. According to leaflet No. 365, this displaces an element within the condenser without changing the position of the upper lens. Therefore, as with the Beck form, the immersion contact with the under surface of the slip bearing the specimen is undisturbed.

§ **189. Superstage dark-field condensers.** — These are dark-field condensers to put on the stage instead of in place of the substage illuminator. They are made with the same exactness as the substage form, indeed, in some the optical part is exactly the same as for the substage condenser. In its use the bright-field substage condenser is turned aside and the light reflected by the mirror directly into the condenser on the stage. The only special difficulty in its use is that in moving the preparation to find a desirable field one is likely to get the condenser out of center, as it is held in place only with the stage clips, or the mechanical stage.

Reichert has overcome this difficulty by attaching arms with pegs to fit into the holes in the stage designed for the spring clips. The sliding joint of the two arms connected with the condenser and the pegs is clamped when the condenser is centered, then it is almost as fixed as the substage condenser, and the preparation can be moved freely without much danger of decentering the stage.

§ **190. Immersion contact of condenser and glass slip for high apertures.** — As shown in § 124 for the bright-field condenser, and as indicated in the diagrams (fig. 73) showing the aperture of light that is required to fill the hemisphere above the condenser with light, if a high aperture is required, it can only be obtained by making immersion contact between the upper face of the condenser and the lower face of the glass slip bearing the object to be studied.

As will be seen by the different figures in this group the numerical aperture that can illuminate the object is limited by the refractive index of the medium in contact with the upper face of the condenser.

A general and complete statement is that the aperture of light which can be concentrated upon an object is limited by the medium of lowest refractive index between the condenser and the object, hence the advice to use homogeneous immersion.

As in the reflecting condensers, practically no light can escape from the condenser which has an aperture greater than 1.00, objects mounted in air require immersion contact of the mounting slip and the top of the condenser, although this would be unnecessary with refracting condensers (§ 178). If in air or water, it would seem that water immersion of the slip and condenser would suffice; if the object were in glycerin, then glycerin immersion, and if in Canada balsam or other medium of the refraction of glass, then only would the homogeneous immersion seem to be necessary (fig. 73).

It is advocated, however, that in all cases the homogeneous immersion be made with the condenser for two reasons: (1) If there is homogeneous contact between condenser and slip, not so much light will be lost by reflection from the lower face of the slip. If not immersed, this loss is very great from the obliquity of the light from the condenser to the slip. (2) It is advantageous to use the homogeneous immersion contact because some of the objects mounted in air rest directly on the glass slip, as is also the case with any fluid mounting medium. Being in contact with the glass slip the light passes directly from the glass into it up to the aperture of the index of refraction of the object; hence the object in optical contact with the glass slip receives a greater aperture of light than the surrounding medium could, and is therefore more brilliantly illuminated, as the illumination is as the square of the aperture. One can see the importance of this consideration by a very simple experiment: Clean the top of the condenser thoroughly, then illuminate as strongly as possible. The top of the condenser will remain relatively black. Scatter a few grains of flour or powdered starch upon the face of the condenser and those that are in optical contact with the condenser will be very white from the light passing from the condenser to them, and being reflected by them (fig. 76B).

§ **191. Slips and cover-glasses for dark-field work.** — As most dark-field condensers have a fixed focal distance, and the object must be placed in the focus to be properly illuminated, it follows that one must select glass slips of the thickness to bring the objects mounted upon them at the level of the focal point. Slips 0.05 less and 0.05 more than the standard are permissible with most dark-field condensers. The makers mark upon the condenser mounting the thickness of slip to use, hence one can select those of the right thickness by the use of micrometer calipers (figs. 219, 220). Dealers in microscopical supplies will also furnish slips of the required thickness. As these measured slips are often used over and over it is better to have them of a

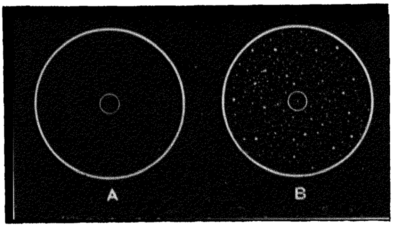


FIG. 76. TWICE ENLARGED UPPER FACE OF A FULLY-LIGHTED, PARABOLOID, DARK-FIELD CONDENSER TO SHOW THE SMALL CENTERING CIRCLE.

A Glass surface perfectly clean.
B Starch granules in optical contact with the glass.

permanent glass. Those of a slight greenish tinge now made in America are much to be preferred over the white, unstable glass so common in the market.

Cover-glasses should be about 0.15 to 0.18 mm. in thickness. That is, they should not be thicker than the working distance of the highest objectives to be used.

§ **192. Cleaning slips and covers for dark-field work.** — From much personal experience the writer urges all who are to undertake serious work with the dark-field microscope to use the method of Stitt for cleaning the slips and covers. This method is given in full in §§ 512, 515.

§ **193., Test preparations for the dark-field.** — As the most perfect effects are obtained with some difficulty, it is advised that preparations be made with slips and covers of known thickness by which the performance of the dark-field condenser can be tested.

Suppose the thickness of slip to be used with a given condenser is 1.30 mm. Select a slip of exactly 1.30 mm. and a cover-glass of 0.15 mm. in thickness. Clean well. In the middle of the slip write the thickness with thin white ink. A delicate brush is good for this. Some parts should appear white to the naked eye and others must be very faint. After the ink is dry, add a drop of Canada balsam and put on the cover-glass and press it down well. The particles of which the white ink is composed serve well for deflecting the light.

When ready to undertake the study of some preparation, if this standard is used to get the optimum lighting, one can feel confident that the conditions are favorable for the object to be investigated. This may seem like too much trouble, but no trouble is too great to enable one to get the best possible results if the work is worth doing at all.

§ **194. Determining the thickness of slip for the best results with a dark-field condenser.** — The writer has not always found the thickness of glass slip recommended by the makers of the apparatus the most perfect for that particular condenser. Indeed, sometimes the results obtained by using the recommended thickness of slips were very imperfect.

With dark-field condensers of the cardioid form which give a sharp focus, the proper thickness of slide to use for the best results can be readily found by the use of slips of various thickness ground on one face.

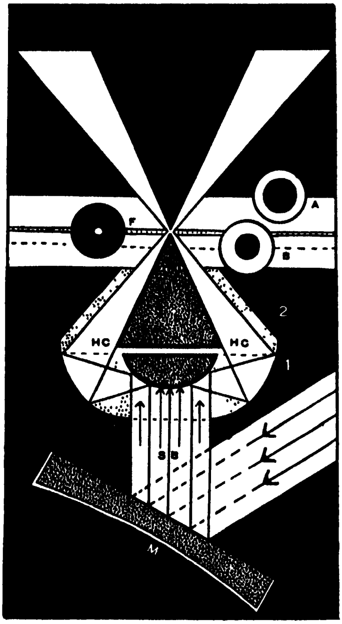


FIG. 77. BISPHERIC DARK-FIELD CONDENSER.

SB Solid beam of light from the mirror (*M*) to the central stop, which cuts out all but the border rays.

1, 2 Lower and upper elements of the condenser. There is reflection from the lower element only. Compare with the cardioid form (fig. 84), where there is reflection from both elements.

HC Hollow cone of light.

F Slide of the thickness to bring the object in the focus of the hollow cone.

A Slide too thick, bringing the object above the focus. There will be a dark central spot in the field.

B Slide too thin. This will locate the object below the focus in the hollow cone and give a dark spot in the field.

To determine the thickness the smooth glass surface is connected with the top of the condenser by homogeneous liquid. The microscope is brilliantly lighted by an arc light or by one of the lamps used for dark-field observation (fig. 80). Of course sunlight can also be used. Employ a low objective and low ocular. Focus the spot of light on the top of the glass slip. As it is much too thick, there will appear a ring of light with a black center (fig. 77). If one uses thinner slips, the bright spot on the top of the slip will grow smaller and smaller and the central black spot disappear. The slip showing the smallest bright spot is the one of correct thickness, for it brings the focus of the hollow cone of light from the condenser at the surface of the slip where the object to be examined is situated. If one uses a slip much thinner than required, there will also be a ring of light with a dark center (fig. 77). For measuring the thickness of the slips a micrometer caliper is used. For grinding the surface, a piece of plate glass or other smooth, flat glass is used for the grinding surface. On this is placed some very fine carborundum or emery flour, and a small amount of water added. The glass slip is then put down on the grinding powder and rubbed around on the plate glass. After a little experience one can grind one face of a glass slip in 20 to 30 seconds. After being ground the surface is well washed with water and wiped dry. For use, the unground surface of the slip is connected with the top of the condenser by homogeneous liquid. The ground surface will then be up and it serves to show the ring or spot of light from the condenser exactly as the ground glass in a photographic camera shows the image. A ground glass slip serves also to aid in centering the condenser (§ 202).

In practice one must be sure that the glass slip is pressed down in close contact with the top of the condenser. If there is a considerable stratum of the homogeneous liquid between the slip and the condenser the thickness of the slip is thus virtually increased in thickness and one would conclude that a thinner slip was needed, which would be wrong. If one works in a warm room and uses an immersion liquid which is not too thick, it is easier to make proper contact between the slide and the top of the condenser. This is taken advantage of when by necessity or mistake an object has been mounted on too thin a slip. By using plenty of the immersion liquid between slip and condenser the condenser can be lowered sufficiently to bring the focus at the level of the object on the upper surface of the thin slip.

LIGHTING AND LAMPS FOR DARK-FIELD MICROSCOPY

§ **195.** A glance at figures 70, 74 and 84 will give a clear notion of how little of the light passing through the condenser is deflected by the object into the microscope, consequently the source of light must be of great brilliancy or there will not be enough to give sufficient light to render the minute details of the objects visible, when high powers are used. This visibility of minute details involves three things: (1) The aperture of the objectives; (2) The aperture of the illuminating pencil; (3) The intensity of the light.

The most powerful light is full sunlight. Following this is the direct current arc, the alternating current arc and then the glowing filament of the gas-filled or mazda lamps, and the "pointolite, or tungsarc" lamps.

The reflecting condensers are designed for parallel beams, consequently the direct sunlight can be reflected into the condenser with the plane mirror of the microscope. If the arc lamp, a mazda lamp, or any other artificial source is used, a parallelizing system must be employed. The simplest and one of the most efficient is a planoconvex lens of about 60 to 80 mm. focus with the plane side next the light and the convex side toward the microscope mirror (fig. 81), i.e., in position of least aberration. This is placed at about its principal focal distance from the source whether that be arc lamp, mazda lamp, or any other source and the issuing beam will be of approximately parallel rays. These can then be reflected up into the dark-field condenser with the plane mirror.

In dark-field work it is wise to recall that it is somewhat like studying the planets and the moon, that is, one is observing objects which are seen by light deflected by them, hence it is better to work in a dark or dimly lighted room so that the comparatively mild light from the object shall not be rendered obscure or misty by an admixture of scattered light reaching the eye at the same time.

§ **196. Direct sunlight.** — The early workers, Wenham, Shadboldt, Stephanson, Edmunds, Quekett, Carpenter, etc., advocated the use of full sunlight, and that is the best light for the most difficult demonstrations. For continuous observation one must use a heliostat to keep the light in position. Unfortunately, also, clouds are likely to obscure the sun, and night is sure to come before the study is completed. Hence artificial sources of light are utilized. For many purposes sunlight is too brilliant, but that difficulty is easily overcome by the use of one or more pieces of ground glass between the sun and the mirror.

§ **197. The arc lamp.** — Next to the sun the arc lamp gives the most brilliant light. Small forms have been devised using 4 to 6 amperes of current (fig. 78). The direct current arc is most satisfactory for there is but one brilliant crater supplying the light. The alternating arc has two equally brilliant craters, and the double light is not easy to utilize.

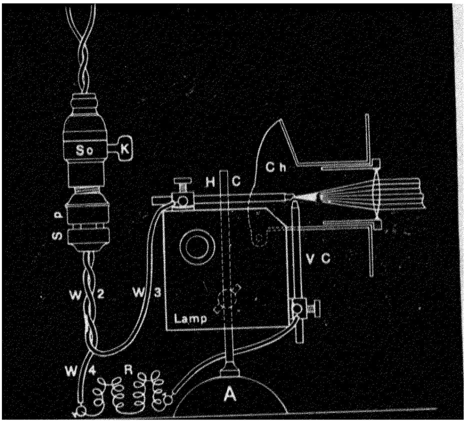


FIG. 78. SMALL ARC LAMP FOR DARK-FIELD ILLUMINATION AND FOR PHOTO-MICROGRAPHY.

A Support for the lamp; *H C*, *V C*, The horizontal and vertical carbons. With direct current the upper carbon is made positive and the lower one negative.

Ch The hood and sleeve to cover the crater and contain the parallelizing lens.

R The resistance or rheostat.

W 2, 3, 4 Wiring for the arc lamp.

So, *K S p* Socket with its key-switch and separable plug below.

Like sunlight, the arc is brighter than necessary for much of the work with the dark-field microscope. It can be softened to the desired brightness by pieces of ground glass between the lamp and the microscope.

§ **198. 6-Volt headlight lamps.** — Next to the arc light, and far more satisfactory to use is a 6-volt headlight lamp. This has a very small, closely coiled filament or a band filament giving a source not much larger than the crater of the arc lamp and hence closely approximates a point source of light. The brilliancy is also very great as the filament is at about 2800° absolute. The two sizes that have been found most useful are the 72-watt and the 108-watt bulbs.

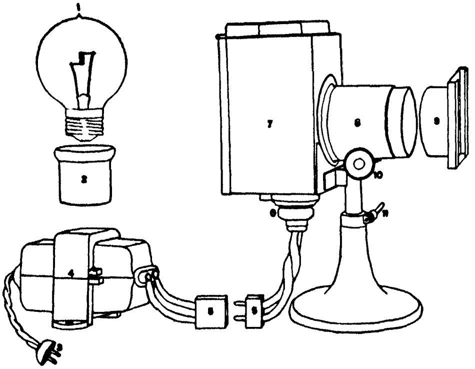


FIG. 79. ADJUSTABLE 6-VOLT, 108-WATT LAMP FOR BRIGHT-FIELD AND FOR DARK-FIELD ILLUMINATION.

1 Coiled filament, 6-volt lamp.
2 Mogul base.
3 Connection for the 110-volt circuit.
4 Step-down transformer, i.e. 110 to 6 volts.
5-5 Mistakeless connection for the lamp.
6 Mogul socket in the lamp-house.
7 Lamp-house.
8 Tube with condenser.
9 Screen carrier to attach to the condenser tube.
10-11 Adjusting screws for tipping the lamp, and raising and lowering it.

(Modified from the Catalogue of the Bausch & Lomb Optical Co.)

In use these lamps require some means of reducing the voltage from 110 or 220 to 6 volts. A rheostat is sometimes used, but this is exceedingly wasteful. If the current is alternating, a step-down transformer accomplishes the reduction with practically no loss. As the wattage is the voltage into the amperage, it follows that the transformer in reducing the voltage from 110 to 6, must raise the amperage a corresponding amount. Then the 72-watt lamp with a 6-volt current requires $\frac{72}{6}$ or 12 amperes, and the 108-watt lamp $\frac{108}{6}$ or 18 amperes of current.

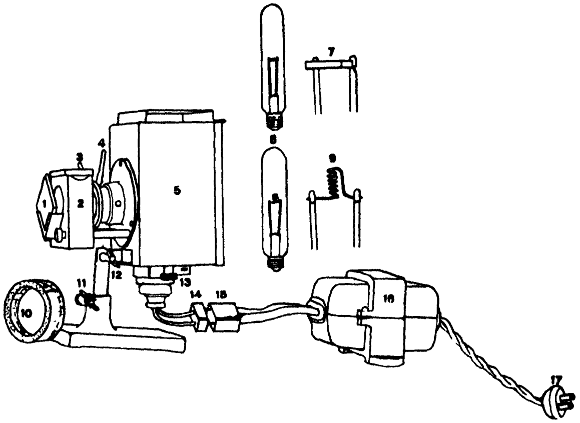


FIG. 80. ADJUSTABLE, 6-VOLT, 108-WATT RESEARCH LAMP.
(Modified from the Bausch & Lomb Optical Co.'s Catalogue.)
1 Daylight-glass screen.
2 Frame for holding water cell.
3 Handle for opening and closing the iris diaphragm.
4 Handle for focusing the condenser.
5 Lamp-house.
7 Ribbon-filament of the upper cylindrical lamp.
8–9 Coil-filament and cylindrical lamp.
10 Water cell of a glass tube with heat absorbing faces (Dr. H. P. Gage).
11–12 Set screws for the inclination and elevation adjustments.
13 Adjusting screws for centering the lamp.
14–15 Mistakeless connection for the lamp cable.
16–17 Step-down transformer (110 to 6 volts), and connection for the 110-volt circuit.

The heating of the lamp filament depends upon the amperage. It is also to be remembered that the greater the amperage, the larger must be the wire conducting the current. Hence the wire from the transformer to and from the lamp must be of much larger size than the wires to and from the transformer to the 110-volt circuit of the ordinary lighting system.

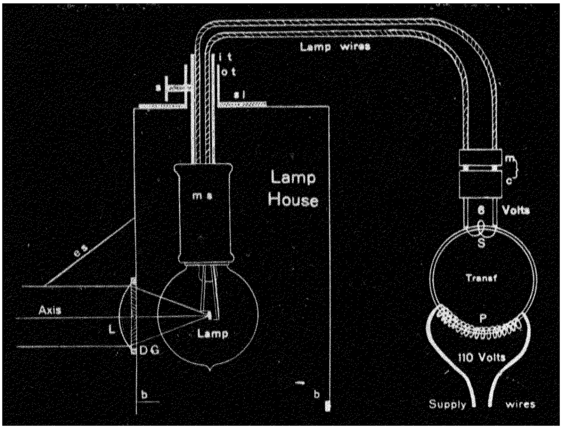


FIG. 81. DIAGRAM TO SHOW THE CONSTITUENT ELEMENTS OF THE 6-VOLT, DARK-FIELD LAMP AND TRANSFORMER.

Supply wires from the 110 volt-circuit to the primary (*P*) side of the transformer with its many coils.

Transformer to step the voltage down from 110 to 6.

P Primary side of the transformer with many coils.

S Secondary side of the transformer with few coils.

6 Volts The number of volts in the wires to the lamp but as the voltage is stepped down the amperage is proportionally increased to hold the wattage constant.

M C Mistakeless connection. A connection which prevents joining the lamp wires with a 110-volt circuit.

Lamp wires These must be heavy to carry the high amperage.

D G Polished daylight glass, and *L* parallelizing lens.

Ordinary cable used on desk lamps, etc., is plenty large enough to carry the current to the transformer, but from the transformer to and from the lamp the conductor should be much larger. Heater cable has been found good, especially if a double cable is used as shown in the diagram, (fig. 81.) To avoid mistakes in connecting the lamp and transformer there should be a connection wholly different from that connecting the transformer to the ordinary lighting circuit. If the 6-volt lamp is connected directly with the 110-volt lighting circuit, the lamp will burn out almost instantly.

The transformers for use with the dark-field microscope lamp should be substantial and designed for continuous use. Furthermore, in introducing them into the circuit between the lighting system and the 6-volt lamp, one must be sure to connect the 6-volt side or wires with the lamp and the 110-volt side with the lighting circuit. If the transformer is reversed the voltage would be *stepped up* a corresponding amount and the fuses in the lighting circuit burned out.

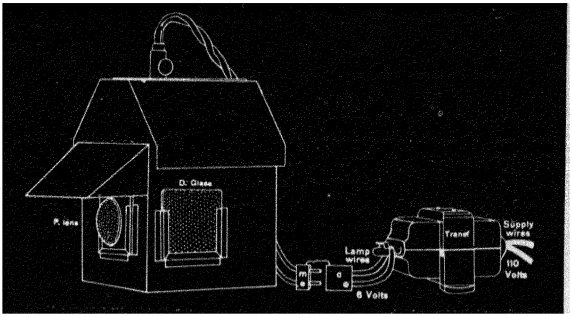


FIG. 82. ORIGINAL 6-VOLT LAMP FOR DARK-FIELD AND BRIGHT-FIELD ILLUMINATION.

In this lamp the daylight window for bright-field illumination is on the side.

The transformers are clearly marked on the two ends either by the voltage, or as they are often used for ringing door bells the end for the circuit is marked " line," and that for the bell is marked " bell." The " bell " wires are the ones to connect with the lamp. If one looks at the wires, it will be found that those on the 6-volt side are much heavier than those on the 110-volt side, for they must carry 12 to 18 amperes, while the wires on the lighting circuit side (110-volt side) have to carry less than 1 ampere. The transformers sent out with the 6-volt lamps are usually so wired and their connectors so arranged that there is no chance of mistake.

§ **199. Chalet microscope lamp (fig. 83).** — The Chalet Microscope lamp, while not designed for dark-field work, answers fairly well for the lower powers, i.e., up to objectives of 8 mm. (20x) used either with a refracting or a reflecting condenser. If it is to be used for the highest powers, it is better to remove one of the daylight glasses. In all uses of this lamp where there is no bull's-eye condenser the lamp should be brought up close to the microscope. If a bull's-eye lens is used with the lamp the distance may well be 20 to 30 centimeters. If the lamp is used without the daylight glass in the window, that is, with the lamp bulb direct, one often gets better results with the concave mirror, as that tends to make the rays from the lamp more nearly parallel. If the ground daylight glass is used or if the naked lamp is used with a bull's-eye lens, the plane mirror is more effective.

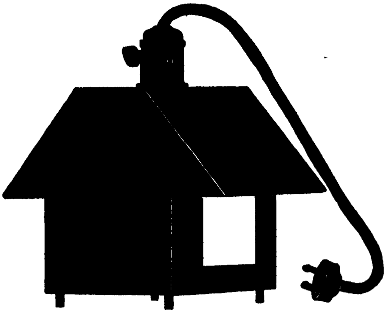


FIG. 83. ORIGINAL CHALET DAYLIGHT MICROSCOPE LAMP WITH TWO WINDOWS.

§ **200. Dark-field condensers with small attached lamps.** — In order to meet the needs of those who can have very limited space, and therefore require minimum bulk of apparatus, substage condenser lamps of small size are connected directly under the condenser. These give fair results, but are not satisfactory as a laboratory instrument, for the light is not brilliant enough to meet the varied demands made in a laboratory, and for research on difficult subjects.

§ **201. Blackness of the dark-field and intensity of the light source.** — A perfect dark-field condenser would give a perfectly black field with any source of light. Tests made on ten different forms with the arc lamp and uranium glass have shown that in all forms a certain amount of the light from the source does pass into the microscope without being directed by the objects in the field of view. This tends to render the background grayish, instead of leaving it perfectly black as it would be if absolutely no light entered the microscope except that deflected to it by the object. The amount of this adventitious light increases with the brilliancy of the illumination, even when the condenser is perfectly centered, the correct thickness of slip used and the lamp and microscope mirror in the most favorable position.

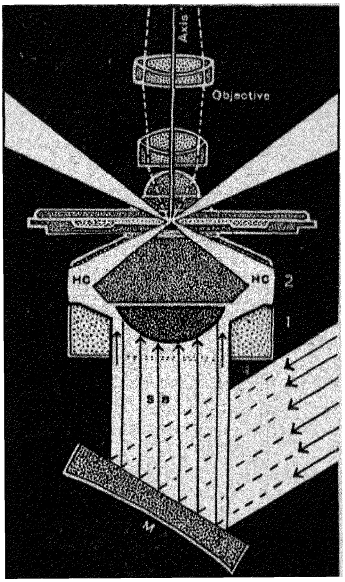


FIG. 84. CARDIOID REFLECTING DARK-FIELD CONDENSER.

(Slightly modified from the 1941 catalogue of the Bausch and Lomb Optical Company.)

SB Solid beam from the mirror (*M*). Only the border rays of great aperture pass on to form the hollow cone (*HC*).

1, 2 Lower and upper elements of the condenser. In the cardioid form both elements reflect. Compare with the bispheric forms (figs. 74, 77) where the reflection is only from the lower element.

With all forms of dark-field condensers the background may be rendered darker by lessening the intensity of the light either by using a weaker light or by putting in the path of the beam from the lamp one or more sheets of ground glass. These ground glass sheets are conveniently held in wooden blocks, then they can be placed anywhere in the beam of light. The closer to the microscope mirror, the more brilliant the light. If the unmodified ground glass subdues the light too much, the ground surface may be oiled, and most of the oil rubbed off with a clean cloth.

With the paraboloid condensers (fig. 84) the field can also be made darker by closing the iris more or less. This is because in closing the iris the outside part of the ring of light entering the condenser is blocked. A glance at the figure will show that in closing the iris the outside rays that are excluded are those which, after the single internal reflection by the condenser, become the inside rays at the least aperture. The rays of greatest aperture are left to illuminate the object under the microscope. With the cardioid forms of condenser with two internal reflections, closing the iris would exclude the most oblique rays. This would darken the field, but at the expense of the most favorable part of the light for dark-field illumination.

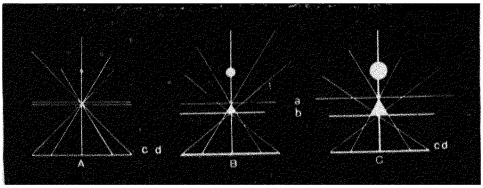


FIG. 85. FACE AND SECTIONAL VIEWS OF THE FOCUS OF THE HOLLOW CONE OF LIGHT FROM DARK-FIELD CONDENSERS.

A Sectional view of an optically perfect dark-field condenser in which the sun is represented as focused nearly to a point. No such condenser exists.

B Sectional view of a possible condenser focus. It is drawn out somewhat and spreads laterally. The variation in the thickness of slide which might properly be used is shown by the two parallel lines enclosing the elongated focus.

C Sectional view with a still more elongated focus. The parallel lines show that the variation in thickness of slide permissible is correspondingly increased.

The apparent size of the sun's image is shown on the axis above in each case. It is least sharp in *C*, and represents fairly the paraboloid condenser where the different zones have different foci, and hence permit of considerable latitude in thickness of glass slip, and give a large, lighted field.

c d The white line above the letters *A B C* is at the level of the top of the condenser.

a b The vertical elongation of the focal point. It is very marked in *C*.

It is to be remembered that any device for darkening the field — except of course the correct arrangement of the apparatus and lamp — makes the object less brilliant at the same time that it darkens the field; and it is the intensity of the light that determines the smallness of the object which can be seen. This is easily demonstrated by using a preparation like fresh blood with very fine elements (fibrin filaments, chylo-microns, etc.), (fig. 89). With a brilliant light all the elements can be clearly made out. Insert one or more sheets of ground glass in the path of the beam from the lamp and the smallest elements seem to disappear. That is, as shown in fig. 67, minute details may be swamped by the excessive surrounding light, and they may also be obliterated if the light from them is so faint that not enough reaches the observer's eye to make them visible.

EXPERIMENTS WITH DARK-FIELD CONDENSERS

§ 202. Centering a substage, dark-field condenser. — While centering the condenser and objective is important with a bright-field condenser, it is far more important if one is to realize anything like the perfect images that are possible with a dark-field condenser. To render centering certain and easy, most makers put a small ring in the middle of the upper face of the dark-field condenser (fig. 76). The ordinary bright-field condenser is removed from the substage, and the dark-field inserted in its place. It must be possible to raise the condenser so that it is at the level of the upper face of the stage, otherwise it cannot be brought close to the glass slip. When it is in position, the iris diaphragm of the substage is opened widely, and also that of the condenser if one is present. For centering it is advantageous to use a low ocular, and a low objective, say one of 50 mm. (3x), then the end of the condenser can be seen as a whole as well as the little ring. The condenser is lighted as strongly as possible and the upper face brought into focus by the coarse adjustment. If well lighted and clean, the centering ring will appear as a shining circle for it allows some of the light to escape from the condenser and scatters it in all directions. Now with the special centering screws of the condenser or with the substage centering screws, change the position of the condenser until it is exactly in the middle of the field of the microscope. It should then be in a favorable position for all powers, although as the different objectives may not have their centers in exactly the same line, some further slight adjustments may be necessary.

§ 203. Special methods of finding the centering circle of the condenser. — Wipe the end of the condenser with clean gauze, using also xylene if necessary to remove any cedar or other immersion oil. Raise and tilt the lamp used for dark-field work till the beam crosses the condenser face at right angles. The little centering ring usually appears with much brilliancy. Often when the ring does not show with the light coming from below it will appear if the end of the condenser is made thoroughly clean.

Another excellent method of centering the condenser whether there is a small centering circle or not is to employ a slip of the proper thickness with one ground face (§ 179). The unground face is put in immersion contact with the condenser and the light directed up through the condenser by the mirror. One can see with the naked eye when there is a bright spot on the ground surface of the slip. Look into the microscope and tilt the mirror in various directions until the smallest, brightest spot is in the middle of the condenser face. The condenser and this spot of light are then put exactly in the middle of the field of the microscope by means of the centering screws. By this method both condenser and light are centered at the same time. See the following section.

§ 204. Centering the light for the dark-field microscope. — If an arc lamp, the 6-volt headlight lamp or tungsarc is used it should be about 25 centimeters from the microscope, and the lamp-condenser should be in a position to give practically parallel light. This can be brought about by focusing the arc crater or the lamp filament on a white wall 5 to 10 meters from the lamp. One can work at night for this adjustment if the room cannot be darkened. When the position is once determined, a mark should be made on the lamp so that it can be adjusted at any time. If the chalet lamp or one having no condenser is used, the lamp should be as close as possible to the microscope.

Arrange lamp and microscope so that the light strikes the middle of the mirror. Use a ground slip of the proper thickness for the particular condenser. Connect the unground face to the condenser with homogeneous liquid, turn the mirror until the light passes up through the condenser and forms a spot of light on the ground face of the slip. Look into the microscope and focus the spot of light. Turn the mirror until the smallest and brightest round spot is obtained. This should be in the exact middle of the upper face of the condenser. One can also see whether the condenser is accurately centered to the objective (§ 202).

If a suitable preparation like fresh blood (§§ 211–212) is substituted for the ground glass and a homogeneous objective used in place of the very low power, the elements of the blood should appear with great brilliancy. Sometimes a slight change in the mirror will increase the brilliancy.

§ **205. Focusing the dark-field microscope with immersion objectives.** — The object on a slip of proper thickness is put in immersion contact with the condenser and a drop of immersion liquid is put on the cover-glass in the middle of the preparation. The mirror is arranged so that the light shines in the drop of immersion liquid on the cover. The objective is then focused down until it touches the immersion liquid. When this happens, a flash of light will be seen if one works in a dimly lighted room and looks toward the lower end of the objective. When one is sure that the objective is in the immersion liquid, look into the microscope. There will be a diffuse bright area or field. This will be the appearance whether the objective is above or below the focus of the object. To make sure that it is not below, focus up slightly. If nothing appears focus down slowly. As the objective approaches the focus of the object the field will be very bright all over. As one continues to focus down the field will become gray and in many cases appear like a bank of clouds; focusing still farther down the field will become darker and darker and finally the field will be dark with the bright objects appearing as if shining by their own light in blank space.

§ **206. Indicator to aid in focusing.** — The above assumes that there are enough particles so that there will be some in every field. In case a liquid is being examined where there are few particles, and therefore the possibility of blank fields, it is a good plan to make a faint x with a red glass pencil on the middle of the slip before the preparation is added. One can then focus on the red cross and feel sure that the objective is at the right focus to give an image of any particles which may be in the preparation.

§ **207. Objects suitable for the dark-field microscope.** — Fresh preparations: Any of the body fluids, — saliva, milk, blood, chyle, lymph, pleural, pericardial, peritoneal, and all other normal body fluids, secretions and excretions; all pathological fluids and the body fluids in pathological conditions of the body; isolated elements of the tissues of the body.

In biology, use all the body fluids of plants or animals and their isolated tissue elements and for minute organisms — microbes — the entire organism (bacteria, protozoa, etc.). For the most satisfactory results the elements must be scattered so that there will be blank space between them. If they are so numerous or so close together that the whole field is filled with light from them, the benefit of contrast is lost. Ordinary thick microscopical sections are therefore not suitable. Very thin sections, stained and unstained, may be used for the difference of refractive index is sufficient to give differentiation, and if the differential stains used are fluorescent, this will help in the differentiation.

Dr. Chamot has pointed out that the dark-field is of great help in the study of foods, fibers, crystallization phenomena, sub-microscopic particles and colloids. He adds further (pp. 35-37): "This method is invaluable for demonstrating the presence of very minute bodies or those whose index of refraction is so nearly the same as that of the mounting medium in which they occur as to cause them to escape detection when illuminated by transmitted light," that is, the ordinary light used for bright-field microscopy.

§ **208. Mounting fluid preparations.** — Take saliva for a trial specimen. The tongue is rubbed around on the gums and cheeks and the saliva brought to the lips. Some of this saliva is transferred by a clean toothpick to the middle of a suitable glass slip. A cover-glass is put over it and pressed down moderately. If any liquid is pressed out, it is wiped away with a piece of gauze. Then with a fine brush the cover-glass is sealed by painting a ring around the edge of the cover. For the sealing one can use some thick oil like castor oil, or thick automobile oil or shellac.

With watery preparations like saliva the cover-glass is likely to be dragged around in moving the slip for studying different parts, because of the viscosity of the immersion liquid. The shellac cement dries quickly and anchors the slip firmly.

Some workers prefer the immersion liquid made of heavy white petroleum mixed with alpha-bromo-naphthalene because it is not so sticky as cedar oil.

In case very few particles are present in the liquid to be studied, remember to make the faint red cross on the slip to aid in focusing (§ 206).

§ **209. Preparations to show spirochætes.** — (*a*) Those of the mouth, *Spirochæta bucalis:* Especially around the base of the teeth next to the gums in most adult human beings there are spiral micro-organisms which show the cork-screw form and movements of that group of organisms with great clearness. Those from the mouth are large enough and numerous enough to make them easy of observation and thus to gain some adequate notion of the characteristic appearance and movements of spiral organisms (*Treponema*, etc.).

For making the preparation use a fresh toothpick and collect some of the material around the base of the teeth and put it on the middle of a suitable glass slip. Add a drop of saliva, and put on a cover-glass and seal the cover (§ 208).

Focus as described in § 205, and in the field will be found salivary corpuscles, and large epithelial scales from the lining epithelium of the mouth, minute particles, bacteria, motile and stationary, and the cork-screw-like buccal spirochætes. It is of great help to get thoroughly familiar with the appearance and movements of these apparently harmless spirochætes in preparation for the detection of the spirochætes of Vincent's angina and those of syphilis.

(*b*) *Spirochæta pallida; Treponema pallidum* of syphilis. — This spiral micro-organism discovered by Schaudinn in 1905 in the lesions of syphilis is rather difficult of demonstration by any method, but most easily shown by the dark-field microscope in fresh preparations. The use of the dark-field microscope for such demonstrations has had great influence in bringing the possibilities of the dark-field to the attention of the medical profession, and has stimulated the microscope makers to make convenient and efficient lamps and condensers for general use. The biologist has thus put at his service a powerful aid in pure research. Wenham, Edwards and many others years before had shown how clearly living microbes could be demonstrated by its help, but it was the practical application that brought the dark-field microscope into general use.

The method of making an examination for diagnostic purposes here given is compiled from Stitt, Thro, and from personal observations in a clinic held in the office of Dr. M. A. Dumond.

Slips and covers are first carefully cleaned and placed in a convenient position. Then the suspected lesion is rubbed with a match stick or a toothpick around which has been wrapped some cotton or a strip of gauze. The rubbing is continued till the covering is removed and the lymph commences to exude. Stitt recommends that the lesion be first washed with alcohol and dried with cotton or gauze. In a few minutes, (3–5), a clear lymph will exude. A drop of normal saline solution is placed in the middle of a slip, and with a pipette or a platinum loop some of the serum is transferred to the salt solution and mixed with it. Then the cover-glass is added. Or the cover-glass is touched to the exuded serum and then put over on the slip with the salt solution. The edge of the cover-glass can be sealed with oil or preferably with shellac so that it will be anchored firmly and not be displaced when moving the slip around, due to the viscosity of the immersion liquid between it and the objective. The organisms are sometimes few, requiring much searching, hence the microscope should have a mechanical stage to aid in a systematic search. If present, and the specimen is examined soon, the active movements of the spirochætes will help in their detection.

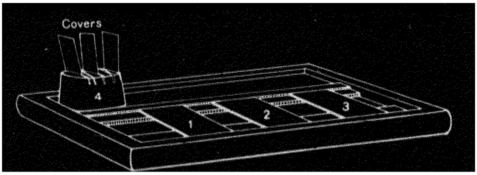


FIG. 86. SMALL TRAY WITH CLEANED SLIPS AND COVER-GLASSES.
The covers are set up in grooves in a cork so that they can be easily grasped by the edges.

After becoming familiar with the appearance of the organism they are easily detected when motionless. In the preparation there will be many minute particles undergoing the Brownian movement. There may also be present blood corpuscles, white and red. The spirochætes are so characteristic in form and movement that there should be no confusion.

§ **210. Infusoria and other micro-organisms in ditch-water infusions.** — A world of interesting forms can be easily obtained for study with the dark-field microscope with all powers by getting some water from a long established ditch or pond, and adding to it some of the grass along the edge of the water. If this mixture is kept in a warm room the organisms will multiply with amazing rapidity. If some of the scum, scrapings from the plants or mud on the bottom is placed on the center of a glass slip it can be studied with the 16 mm. (10x) and lower objectives without any cover-glass. If the Abbe condenser is used with a central stop (fig. 70) below the entire condenser the field lighted will be sufficient for the 16 mm. (10x) objective; if the higher powers are needed then a dark-field element (§ 181) or a paraboloid dark-field condenser works well. The lighted field will be of sufficient size for the lower powers. For both forms it is best to have the slide in immersion contact with the condenser, although with the dark-field element one can get fairly good results without immersing the slide. For the highest powers the reflecting, cardioid dark-field condensers are most satisfactory because of the sharp focus of the hollow cone of light. These must always be in immersion contact with the slide. For all objectives above the 16 mm. (10x) the specimens must be covered with a cover-glass; it is well to seal the cover glass so that it will not move, and so the liquid will not evaporate. A study of such a preparation will give a wonderful insight into the form and activity of these lowly creatures, and reveal a beauty of design that will always be remembered.

§ **211. Fresh blood and the dark-field microscope.** — Perfectly fresh blood is one of the best objects to study by this method. As pointed out by Dr. Edmunds nearly 50 years ago, blood with the dark-field illumination appears like a new object so many things are seen with the greatest distinctness that are wholly invisible or merely glimpsed when examined by the bright-field method.

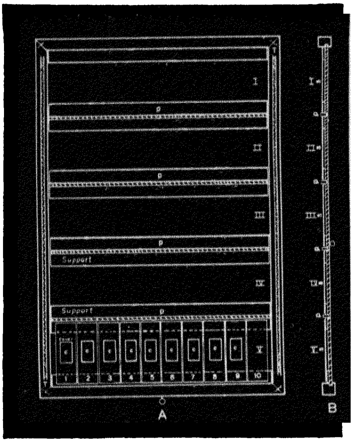


Fig. 87. Large Slide Tray with Supports for the Glass Slips so that the Immersion Liquid on the Under Surface Shall not Touch the Tray.

A Face view of the slide tray.
B Sectional view.

In all experiments the preparations should be preserved till the work is completed, then doubtful points may be cleared up by re-examining earlier preparations.

(1) Carefully cleaned slips and cover-glasses of the right thickness are placed where they can be easily grasped (fig. 86).

(2) For obtaining the blood the part to be punctured is washed with soap and water and then cleaned well with a piece of gauze well wet with 95% alcohol to which has been added 2 grams of mercuric chlorid to the liter (two-tenths of 1%). A needle or Dr. Moore's hæmospast is cleaned by the alcohol also and then the puncture is made. The ball of the middle finger of the left hand has been found a favorable place to get the blood. Tie a piece of gauze around the base of the finger and then squeeze it and a drop of blood will exude. The drop should be fairly large. Now grasp the cover-glass by the edges with the thumb and index of the right hand and touch the cover to the top of the drop. Enough blood will adhere to the cover. Put it on the middle of the cleaned slip and the blood will spread out. It should be pressed down moderately with curved forceps or by a finger covered with gauze, and the blood running out at the edge of the cover should be wiped away or it will run back by capillary attraction. Some parts of the preparation should appear almost transparent to the eye. If it looks red all over, the blood layer will be too thick and not leave enough blank spaces. Seal the cover-glass with heavy oil. The fibrin network will adhere to both

slip and cover and hold the cover in place. No diluting substance is added to the blood.

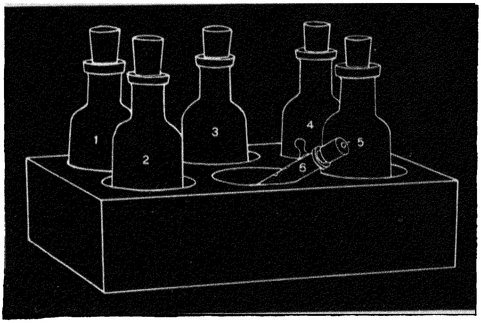


FIG. 88. BLOCK WITH BOTTLES OF REAGENTS AND WITH THE MOORE HÆMOPAST FOR FRESH BLOOD STUDIES.

§ 212. Appearance of blood under the dark-field microscope. — (*a*) The erythrocytes will appear like dark discs with bright rims owing to their convex borders.

(*b*) The leucocytes appear as real white corpuscles owing to the granules within them which turn the light into the microscope. If the room is moderately warm — 20° C or more — the leucocytes, some of them, will undergo the amœboid movement, and the picture they present will be a revelation to those who never saw it or saw it only with the bright-field microscope. From the clearness with which everything can be seen the minutest change can be followed, and also the most delicate pseudopod detected. Another striking feature will be noticed in the moving ones, that is, the vigorous Brownian movement of the granules in the part of the leucocyte with the amœboid movement. In those showing no amœboid movement there is usually no sign of the Brownian movement of the granules; also, if a part of the leucocyte is not undergoing amœboid movement, the particles in it are usually motionless.

(*c*) The fibrin network will be seen like a delicate cobweb between the corpuscles. In different parts of the specimen one can find all the appearances of the fibrin shown in textbooks on the blood (fig. 89).

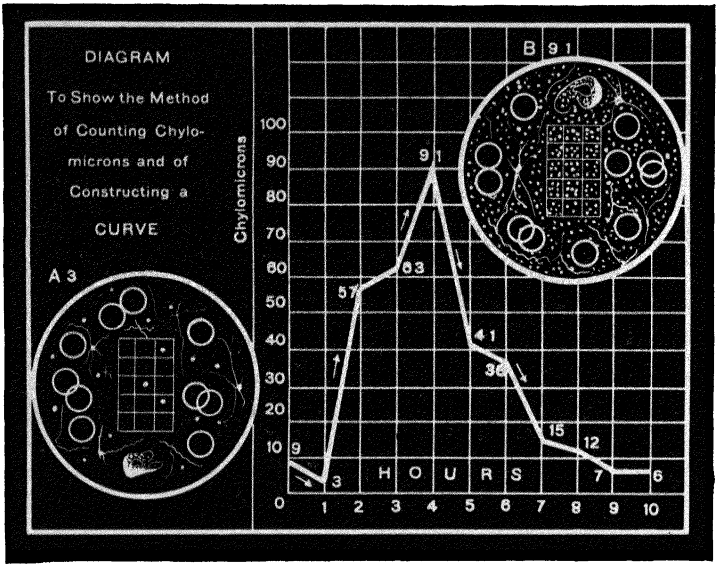


Fig. 89. Two Fields of Fresh Blood to Show the Few Chylomicrons or Fat Particles in Fasting, and their Abundance after a Full Meal with Plenty of Fat.

(From the American Journal of Anatomy, Sept. 1924.)

A 3 Microscopic field in fasting with 3 chylomicrons in the counting net.
B 91 Microscope field 4 hours after a full meal.
The counting net encloses 91 chylomicrons.
The numerals along the curve show the number of chylomicrons during each hour of the digestive cycle of 10 hours.

(*d*) Chylomicrons appear everywhere like bright points in the empty spaces between the corpuscles. They are in every active Brownian or pedetic movement. These chylomicrons will probably be the most unusual part to those studying blood with the dark-field for the first time. The term *Chylomicron* is from two Greek words, χῦλός (chylos), juice or chyle, and μικρόν (micron), any small thing. In modern metrology it signifies the millionth of a meter (§ 380). I have introduced this word to show the origin of these bodies from the chyle, and to indicate their average size. In 1840–1842, Gulliver called these minute granules the "*molecular base of the chyle*" and showed that they were identical in the thoracic-duct and in the blood vessels of the same animal. He gave their average size as 1/36,000 to 1/24,000 of an inch (1μ to 0.5μ). They have been called by others free granules or granulations, elementary particles, hæmokonia, blood dust, etc. (fig. 89).

(*e*) A very striking view of the fibrin network may be obtained by irrigating a thick unsealed blood preparation. If a drop of normal salt solution is placed on one edge of the cover-glass and a piece of blotting paper on the other the liquid is drawn through, washing out many of the erythrocytes. If the washing-out process is watched under the microscope, the erythrocytes will be seen gliding over or through the fibrin network, or some of them will be anchored at one end and if the current is rapid the corpuscles will be pulled out into pear-shaped forms.

The leucocytes look like big white boulders in the stream, wholly unmoved by the rushing torrent around them.

Troubles in Dark-Field Microscopy

§ **213.** If one has available a good light source, a good dark-field condenser, an immersion objective of suitable aperture for the condenser, cover-glasses and glass slips of the proper thickness, and finally suitable objects for study, one soon learns to get good results; but with this instrument the technique is far more exacting than with the bright-field microscope. Troubles which might not be very noticeable with the bright-field microscope will spoil a dark-field image. All of the troubles hereafter mentioned have repeatedly blocked the way of the writer or his pupils, and many others are likely to occur; but the results when they are good are so satisfactory that no one minds the labor necessary when once a good dark-field image of a familiar object is seen.

(1) Lack of a dark background. This may be due to either of the following causes, or possibly a combination of them:

(*a*) The aperture of the objective may be too great for the condenser so that direct light from the condenser enters the objective. This is quite likely to happen from forgetting to insert the proper reducing diaphragm when large apertured immersion objectives are being used. This is a strong reason for the belief that for darkground work, immersion objectives of suitable aperture be furnished by the opticians (§ 185). If the aperture of the objective is near the limit of that of the condenser there is liable to be a halo on one side, especially if the condenser is not perfectly centered to the objective (fig. 90). This halo is more likely to appear with a low ocular having a large field than with a high ocular with a smaller field.

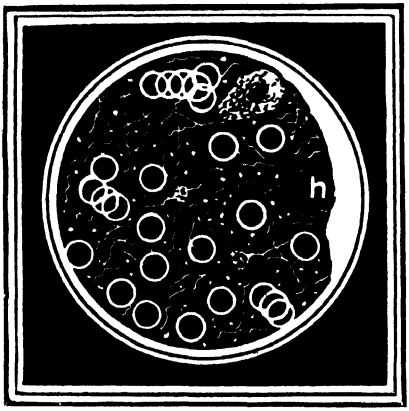


FIG. 90. FIELD IN A DARK-GROUND MICROSCOPE WITH A HALO (*h*) ON THE RIGHT SIDE.

(*b*) There may be an air bubble in the immersion liquid which directs a flood of light into the objective. By moving the preparation this air bubble can be got out of the way, but sometimes it persists in keeping in the way. In such a case it may be necessary to focus the objective up and wipe away the immersion liquid and add a fresh drop. Air bubbles in the immersion liquid between the condenser and the glass slip sometimes cause trouble. These can often be squeezed out by pressing the slip down. In case it does not remedy the evil, lift up the slip and add new immersion liquid.

(2) Impossibility of getting a good image. This has many causes, but one is not infrequent. If not enough homogeneous liquid is used either above or below, when the slip is moved about in searching the preparation, the immersion liquid gets spread out and there is not enough to make good contact between the slip and condenser or between the objective and cover-glass. This condition should be

kept in mind and if it seems necessary, add more immersion liquid. It must be remembered, too, that if the condenser is lowered the stratum of immersion liquid may be broken and leave the condenser unimmersed, or if the stratum of liquid is not broken, it is extended, thus virtually thickening the glass slip. Finally, it sometimes happens that no immersion liquid is put between the condenser and the slip. In that case the preparation cannot be lighted.

(3) Violent or moderate motion of the particles of the preparation when focusing. This is due to the thickness of the preparation. In focusing for different levels in it the end of the objective comes in contact with the cover-glass and presses upon it. Furthermore, the thick preparation lifts the cover up into an unstable position and the pull of the viscid immersion liquid causes it to rise and fall in focusing even though the objective does not touch the cover-glass. The remedy is to make the preparations thinner.

(4) Impossible to get the objective in focus. — This may be due to a cover-glass thicker than the working distance of the objective. Sometimes owing to the dim light in which one works or to inattention, the preparation is put on the stage of the microscope with the cover-glass down next to the condenser instead of up toward the objective. The glass slip is too thick to focus through when using a high power.

(5) There may be too small an opening in the stage. In that case the condenser cannot be raised high enough to touch the glass slip, and no good image can be obtained even though the space between them were filled with the immersion liquid, for that would bring the focus of the hollow cone of light from the condenser much below the object. It would be like using far too thick a glass slip.

In other cases the opening in the stage may be just large enough to receive the condenser top, but leave no room to change its position in centering. The only remedy is to use a different condenser with narrower top or to have the opening enlarged. With some microscopes there is a removable piece which may be taken out thus increasing the size of the opening in the stage.

(6) The substage may not be able to rise high enough to bring the upper end of the condenser at the level of the top of the stage.

There are two ways to remedy this in some cases. The condenser may be screwed up in its sleeve, thus bringing it at a higher level when the substage is run up. The other change is to shorten the stop for the substage by screwing it downward or by removing it altogether. Then one has to be careful and not to get the upper end of the condenser much above the level of the upper face of the stage.

(7) The glass slide may not be in contact with the condenser (§ 194).

SELECTION OF A DARK-FIELD CONDENSER AND A LAMP

§ 214. Dark-field condensers. — Those now available offer considerable choice. In making a selection one should be guided by the work to be done and by the facilities and space at command. If space is limited and facilities few, one of the superstage condensers or one of the substage condensers with attached light might wisely be chosen (§ 189, § 200).

In a biological laboratory in which many different persons are to make use of it on a great diversity of material, one of the paraboloid condensers is recommended. This gives fully lighted fields for objectives of 16 mm. (10x) and higher objectives. It does not require great skill to get fair results. It gives good results with quite a wide range of glass slips, and is not so sensitive to exact centering as the cardioids. In general it will give good results with unskilled workers, and for those really skilled it will give excellent results for all powers of the microscope.

If one wishes to get the most perfect results with high powers, then a condenser of the cardioid form is to be chosen. It gives a very sharp focus of its hollow cone of light, and serves to light the objects at its focus in the most perfect manner, and gives a very dark background. It requires more skill in its use than the paraboloids, but the results obtained with it are worth the extra trouble.

It is not satisfactory for low powers because of the small spot of light it gives; but that is unimportant, because for the low powers the Abbe condenser with a central stop or a dark-field element (§ 181) answers very well.

Those made by the American opticians have been found of the highest excellence, also those made in England and on the Continent. When one of the cardioids is perfectly centered and lighted, the clearness of minute details in suitable structures and in minute living organisms give a certainty to the picture that is unbelievable to a person who has used only bright-field illumination on them.

Of course, it is desirable to have several: one of the paraboloid, one of the cardioid, one of the combined, one of the superstage forms, and one with the dark-field element (§ 181). This is not commonly possible except in laboratories.

Whatever form one possesses it is desirable that it be accurately centered to one microscope, and that that microscope be devoted to dark-field work. It will then always be ready, and one can work with it with the same ease and certainty as with a bright-field microscope.

✓§ **215. Lamp for dark-field work.** — In the selection of a lamp for dark-field work one must also be guided somewhat by circumstances. The best lamp that the writer has used, and he has tested them all, is the 6-volt, 108-watt lamp (figs. 79–82). This gives ample light of daylight quality. It is no trouble, for when once properly connected and arranged, it will go on giving its full light as long as the lamp lasts. It is economical when used with a transformer as it draws only about one ampere from the 110-volt circuit. It is admirably adapted to photo-micrography and many other purposes where a brilliant light is needed. It is available also for bright-field work with the highest powers.

The microscopical training of nearly every worker has been with bright-field illumination, and consequently the appearances given with this form of light furnish the standard. Under the dark-field microscope the same objects have a wholly unfamiliar look, and indeed, as Edmunds pointed out in 1877, seem like new things. It is quite conceivable also that for an investigator who had used the dark-field microscope only, the bright-field image would be equally puzzling. In order, then, to interpret these two images of identical objects, the objects should be studied with both forms of illumination. The more this is done the less unlike do the objects seem, until finally one can interpret the appearance whichever form of illumination is used.

While the combined light- and dark-field condensers (§ 187) are not perfect for either light- or dark-field work they do serve to show the identical object first with one and then with the other form of illumination with only a slight change in the illuminator and no change in the position of the object. As shown in Chapter XIV, §§ 717, 721, this result is also easily attainable with the ordinary refracting condensers.

Of all the reasons for non-success with the dark-field microscope, the most constant one is an inadequate light. Then also the use of unmodified electric light when it is bright enough to bring out the minutest details and particles is very hard on the eyes of the observer unless it is given daylight quality by the use of a daylight glass filter. From an abundant experience of over 10 years with the dark-field microscope the writer is positive that the continuous use of the brilliant, unmodified light would be injurious. Of course, for a diagnosis requiring a few minutes the unmodified light answers, but for continuous study for several hours a day the light should be given daylight quality. This for most observers is not only easier on the eyes but gives a sharpness of detail that cannot be gained by the unmodified light.

As stated above, the 108-watt, 6-volt headlight lamp (figs. 79–80) furnishes abundant light for all uses. The lamp is so constructed that for photography and projection work the full intensity may be used by removing the daylight glass filter. Of course, also, any other filter can be substituted for a special purpose.

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CHAPTER IV

THE POLARIZING MICROSCOPE: OPTICS OF THE MICROSCOPE.

§§ 216–272; FIGURES 91–119

A polarizing microscope is one in which a microscope and a polariscope are combined for the purpose of studying microscopic preparations illuminated by polarized light.

For the biologist the definite information to be gained by using the polarizing microscope may be stated thus:

(1) Whether the object under the microscope is isotropic, i.e., non-polarizing, or anisotropic, i.e., polarizing.

(2) Whether the object is uniaxial or biaxial.

(3) Whether it shows interference phenomena, i.e., a black cross or colors.

(4) Whether the object rotates the plane of polarization.

(5) Whether the object shows pleochroism.

While the full theoretical meaning of all the phenomena may not be clear, the biologist can be certain that the different appearances with polarized light indicate definite physical or chemical properties.

§ **216. Polariscope; polarizer and analyzer.** — A polarizer is an optical device for producing polarized light, and an analyzer is a device to aid the observer in determining whether the object illuminated with polarized light has any effect upon that light.

The polariscope most frequently used with a microscope consists of two prisms of Iceland spar (transparent calcite, $CaCO_3$). The light traverses them lengthwise. The prisms are cut along a diagonal and the cut surfaces polished. The two halves of each prism are then cemented together with Canada balsam or linseed oil.

One prism, the *polarizer*, is placed between the source of light and the object, and serves to polarize the light before it reaches the object.

According to the classical theory, ordinary light from the sun or any other source, is vibrating in all planes transverse to the direction of propagation of the light. When such light enters the calcite prism polarizer it is divided into two parts. One of these parts, called the *ordinary ray*, is refracted most, and meets the cemented surface at an angle greater than the critical angle for calcite in contact with the cementing medium. It is, therefore, reflected to the side of the prism and does not pass on to illuminate the object.

The other ray, called the *extraordinary ray*, is bent less and can therefore pass through the cementing medium and the prism to illuminate the object. Both rays are polarized, but one is eliminated. The object is thus illuminated by light vibrating in one definite plane.

It will be noted that only half the light gets through the polarizer to illuminate the object, hence one must have plenty of light to start with.

Objects illuminated by polarized light look the same as though they were illuminated by ordinary light, except those exhibiting pleochroism (§ 233).

The addition of another Nicol prism, the *analyzer*, so turned that it excludes the light from the polarizer, serves to show whether the object under the microscope has produced any change in the polarized light. This is brought about as follows: In fig. 91 the diagonals of sections of the prisms are parallel. In this position the polarized light passes directly through both prisms. If the analyzer is rotated 90°, none of the polarized light transmitted by the first prism can pass through the second. When so arranged that no light can pass through both prisms, they are said to be *crossed*.

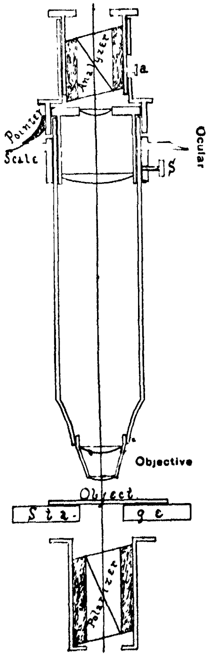

FIG. 91. MICRO-POLARISCOPE IN POSITION ON THE MICROSCOPE.

Polarizer The Nicol prism under the stage of the microscope.

Analyzer The Nicol prism over the ocular.

Stage The stage of the microscope.

Object The object on a slide.

Objective The microscopic objective.

S Set screw for clamping the analyzer to the tube of the microscope.

Ocular The microscopic ocular in position.

Pointer and Scale The graduated ring and pointer to show the amount of rotation.

a Handle for raising and lowering the analyzer to arrange it properly with reference to the eyepoint.

§ **217. Polaroid.** — Instead of Nicol prisms for the polarizer and analyzer a substitute has been recently devised. It is called Polaroid, and is composed of a multitude of fine, elongated crystals imbedded in a cellulose matrix. This matrix is usually mounted between thin plates of glass or heavy safety film.

The polaroid can be used with any microscope and shows the phenomena of polarized light satisfactorily. It is considerably cheaper than the Nicol prism polariscopes. However, for the most accurate work with polarized light it is necessary to have an accurately graduated analyzer, a centering, graduated revolving stage, an ocular with cross-hairs, means for using the quartz wedge, and a selenite plate for red of the first order. Such an instrument is shown in figure 92. (See Martin Grabau, Polarized Light and Its Application, 1940. Polaroid Corporation, Cambridge, Mass.)

§ **218. Isotropic and anisotropic objects.** — An isotropic, singly refracting or non-polarizing body is one which does not divide the beam of light traversing it into two beams vibrating in different planes, but the entering light vibrates in all planes as when entering. Such an isotropic substance is glass, and when it is placed between the crossed polarizer and analyzer the light is not restored. When, however, an anisotropic, doubly refracting or polarizing object is placed between the crossed analyzer and polarizer, it divides the light from the polarizer into two beams and one of them can pass through the analyzer. The object will then glow with a soft light on a dark background. Cotton, linen, and other vegetable fibers are anisotropic, and so are many crystals, and animal tissues like muscle and bone.

TESTING THE POLARIZING MICROSCOPE

Whether the polarizing microscope is simple or elaborate, there are some definite tests that should be made before accepting the results obtained by its use.

§ **219. Testing the polarizer and the analyzer.** — The polarizer is usually as perfect as the manufacturers can make it, and so is the analyzer. It is simple to find out whether the polarizer and analyzer exclude all light when crossed, and allow it to pass when in any intermediate position. For this test, and indeed for all the tests, one should work in a dark room, if possible, so that all extraneous light is absent.

The condenser is removed, also the objective and the ocular.

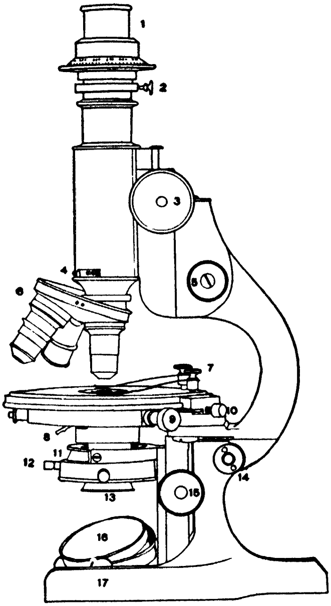

FIG. 92. CHEMICAL MICROSCOPE OF CHAMOT AND MASON
(Outline Drawing from the Catalogue of the Spencer Lens Co.)

1	The analyzer.
2	The set screw to hold the analyzer in place.
3	Focusing wheel for the coarse adjustment.
4	Slit in the tube above the objective for the quartz wedge, etc.
5	Fine adjustment wheel.
6	Revolving nose-piece with three objectives in place.
7	Spring clips for fixing the slide in place.
8	Handle of the iris diaphragm of the condenser.
9	One of the centering screws.
10	Screw for fixing the stage at any position in its rotation.
11	Fork and contained substage condenser.
12	One of the centering screws for the condenser.
13	The polarizer.
14	Nut for fixing the inclining pillar in place.
15	Wheel for the rack and pinion for raising and lowering the condenser.
16	Mirror.
17	Foot of the microscope.

With the polarizer in the zero position and also the analyzer, the tube of the microscope is lowered as far as possible to bring the polarizer and analyzer near together. Then light is directed through the polarizer. If the polariscope is perfect there will be two positions, 0° and 180°, when there will be total darkness. In other positions there will be twilight, and when either element is rotated 45° there will be the brightest light. Apparently it is not possible to make a polariscope so perfect that there is complete extinction of light when the nicols are crossed. Some of the light emerging from the analyzer may not be completely polarized on the one hand; and on the other, when used with various optical parts, the scattering and depolarization of a part of the light by the lenses of the condenser, ocular and objective leave some of the light in a position enabling it to pass the analyzer.

It is also intelligible that, if the light from the source is excessive, even more of it will be in condition to pass the crossed nicols. In any case the field may not be perfectly black when the nicols are crossed in the most perfect instruments. On the principle of contrast, however, the field may appear densely black if there is only a small amount of polarizing material under the microscope. For example, cotton fibers polarize so brilliantly that the field about them appears absolutely black at first. If one moves the slide so that there are no cotton fibers present it will be seen that the field is not black but dark gray. (See Beck, part ii, p. 200; Chamot and Mason, vol. ii, p. 274).

§ **220. Testing the condenser.** — After one is convinced that the polarizer and analyzer are satisfactory, it is well to test the condenser. For this the condenser is put in place above the polarizer and the diaphragm opened wide. The light is then sent up through the polarizer. No object, objective, or ocular should be in place, and the analyzer should be at the zero or crossed position. If the condenser is isotropic, the field will remain just as dark with crossed nicols as it is without the condenser. Sometimes the condenser lenses are under strain, then they become polarizing, and the field cannot be made dark in any position of the polarizer and the analyzer. Such a condenser, although it may be perfectly good for ordinary light observations, should be discarded for polarization experiments, and an isotropic one used.

§ **221. Testing the objectives.** — Many objectives have one or more of the lenses under strain, and therefore anisotropic. With such objectives the field will not be dark when the nicols are crossed, and they are not suitable for polariscopic work, although they may be perfectly good for ordinary microscopic observation. To test an objective, put it in place, cross the nicols and light the microscope well. If the objective is isotropic the field will remain dark with crossed nicols. If the objective is under strain, the field cannot be made dark. Oculars are not so likely to be under strain, but those to be used with the polarizing microscope should be tested in the same way as the objective.

It will be noted in these tests that the position of the analyzer for the most perfect darkness or extinction is very precise. A rotation of 5 degrees either way renders the field gray, and it becomes lightest at 90°, and again darkest at 180°.

§ **222. Testing the glass slips for the polariscope.** — Glass is one of the isotropic substances, but when under strain, it becomes anisotropic. The majority of glass slips and corex glass slips tested have been isotropic, but occasionally one is anisotropic. If the glass slip on which an object is mounted is polarizing, no exact estimate of the character of the object being studied can be determined.

To test the microscopic slips on which objects are to be mounted, place one on the stage of the polarizing microscope and rotate the analyzer. If the glass slip is suitable, the appearance will be exactly as if it were absent, but if no dark field can be obtained when the polarizer and analyzer are crossed, the slip must be doubly refracting, and should not be used for mounting objects for the polariscope.

Dr. Chamot informed the writer that often the slips used in chemical microscopy where they had to be heated over the Bunsen flame, became markedly anisotropic. It is advantageous to have a glass slip which one knows is polarizing so that the appearance may be definitely fixed in mind. To prepare such a slip, heat it all over till it is too hot to hold, then with forceps hold one end in the flame of a Bunsen burner till it is a dull red. Cool slowly by waving in the air. If it happens to break, try another. When it is cool, put the end that had been heated under the polarizing microscope, and cross the polarizer and analyzer. A dark field will not result. Move the slide till a part that had not been heated very hot is in the field. Probably a dark field will result when the nicols are crossed. If so, one can see on the same slip an isotropic and an anisotropic appearance.

§ 223. Centering the revolving stage. — A very practical way to get it approximately centered is suggested by Dr. Chamot: A disc of metal with a very small central hole is put into the opening of the stage of the microscope. One can focus on this, and with the centering screws put the small hole directly under the crossing point of the cross hairs. As the centering disc must fit accurately the opening in the stage, its removal is greatly facilitated by having a segment removed from the edge; then with the finger nail it can be easily lifted up.

The other and the most exact method of centering is to use a clear glass slip, and with a fine pen put a minute dot of ink on the middle of the slide, or one can, with the aid of a writing diamond, make a delicate x on the middle of the slide. This is moved about by the hands until the spot or x is exactly under the crossing point of the cross hairs of the ocular. The stage is then rotated clear around and the spot will again be under the crossing point. Now rotate the stage until the spot or x is farthest from the crossing point of the cross hairs of the ocular. With the centering screws move the spot or the x, half the distance toward the crossing point of the lines in the ocular. Then with the fingers or mechanical stage, move the slide till the spot or x is directly under the cross hairs. Revolve the stage. The centering may not be perfect the first trial, but by continuing one can center the stage so accurately that the object under examination will remain directly under the cross hairs of the ocular for that particular objective. If another is turned in place, the centering may not be quite perfect; but usually it is approximately so. For perfect centering the stage must be re-centered for each objective. The rationale of this proceeding is that the crossing point of the lines in the ocular and the center of rotation of the revolving stage must be put on the same axis. If they are not on the same axis, any spot originally placed directly under the crossing point in the ocular will describe a circle when the stage is rotated, and the spot or x will be at the periphery of that circle. It should be at the center of the circle, that being at the center of rotation of the stage.

THE PURPOSE OF A POLARIZING MICROSCOPE IN BIOLOGY

§ **224. Physical analysis.** — The importance of the physical analysis of animal and plant structures has long been recognized by many workers, but this physical analysis has been carried out only in a desultory way. Now with the exact apparatus at a reasonable cost, and the almost perfect artificial sources of light available, it seems possible to proceed in a systematic manner to determine just what and how much the different minute organisms and the tissues and organs of the higher animals and plants are polarizing. There is certainly a difference in the physical organization of organic as well as inorganic substances when they behave differently in polarized light, i.e., whether they are isotropic or anisotropic. Every hint concerning the intimate structure and arrangement of the materials of biology helps to give a truer insight into their organization. It is believed, then, that the information gained by the polarizing microscope and the ultra-violet microscope will aid in helping to understand the living world. Fortunately this physical analysis can be applied to living as well as to dead matter, and it is independent of the possible changes wrought by the highly artificial staining processes after treatment with varied chemicals.

It is hoped that in the future the definite determination of the physical properties of living and fresh animal and plant substances will be sought for as persistently and faithfully as their staining reactions.

EXPERIMENTS WITH POLARIZED LIGHT

Make sure that the polarizing microscope, and the glass slips used for mounting the objects to be studied are suitable for their purpose (§§ 219–222).

§ **225. Vegetable material.** — The tissues of plants are as a rule more strikingly doubly refracting than those of animals, and are very easy to prepare in the living, fresh and fixed or dried condition. Potato is one of the best objects to begin with, for both the starch and the cellulose walls of the vegetable cells are strongly polarizing. Use a fresh, firm potato. Wash the surface well, then with a sharp knife or a razor blade make a very thin slice, including some of the skin. Mount in water or in normal salt solution. Cover and employ first a 16 mm. (10x) objective, then an 8 mm. (20x) or a 4 mm. (40x) objective. Some of the starch grains are very large, and some quite small. Some will be free in the mounting liquid and many of them will be in the cells of the potato. The cellulose cell-walls will be bright when the nicols are crossed, showing that they are anisotropic. The starch grains, whether large or small, will also be bright and will show a black cross. This will rotate as the analyzer is rotated. This black cross will be met in many cases of doubly refracting bodies, and is one of the characteristic features of starch. If one wishes to study dry starch, it is better to mount it in Canada balsam. In case one finds it difficult to get a thin enough section of the fresh potato, a preparation which will show all the points may be made by scraping the fresh cut surface, and mounting the scrapings in water.

§ **226. Cotton and linen fibers.** — If cotton or linen fibers are mounted in water or in balsam, very brilliant polarization is shown with crossed nicols. Plant tissues, whether fresh or fixed, give good polarization effects, and show with the greatest clearness the minutest layers or strands of cellulose, anisotropic crystals, etc. No one interested in the microscopic structure of plants can afford to neglect this means of investigation.

§ **227. Mounting specimens for both polarized light and ultra-violet radiation.** — For this, mounting slips which are non-polarizing on the one hand, and which transmit ultra-violet radiation on the other, must be used. Such slips are composed of fused quartz or of corex d. glass (fig. 218). The polished fused quartz slips are very expensive, costing from $3.50 to $12.00 each. The corex d. polished slips cost about 75 cts. each. Slips cut from sheets of corex glass answer very well even if not polished. Cut from the sheets, the slips cost about $6.00 per hundred. With ground edges, the cost is considerably more.

§ **228. Mounting media for polarized light and ultra-violet radiation.** — These media, like the mounting slips, must be free from polarization and entirely transparent to the ultra-violet radiation. Canada balsam, cedar oil and many of the ordinary media used for mounting are highly fluorescent in ultra-violet radiation and cannot be used. Fortunately water and normal salt solution are available and suited for fresh specimens. For permanent preparations one may use glycerin. It is slightly fluorescent, but in the thin layers used answers very well. It also mixes with water, which is a great advantage for many specimens. For dry or dehydrated objects it was found that the mineral oil used in medicine, whether of a naphtha or of a paraffin base, is transparent to ultra-violet down to 0.3μ. Its refractive index is high (1.48). It is therefore a fairly good substitute for Canada balsam. It is also good for use in making the immersion contact with the condenser and slip, and for the immersion liquid for the oil immersion objectives. (See table, § 269.) If used for permanent mounting in place of balsam, the cover-glass must be sealed as with glycerin mounts (§ 530).

§ **229. Animal tissues and organs; minute animals with polarized light.** — As with plant life, living and minute animals and living and fresh tissues of animals should be studied with the polarizing microscope and the appearances later studied with the ultra-violet microscope.

§ **230. For living animals,** the water in which they are found in nature is the best mounting medium. For the higher forms, the living and fresh tissues should be mounted in some isotonic solution like normal salt, Ringer's solution, etc. The most perfect isotonic solution is the body juices in which they are naturally bathed in life. It is not easy to get these in all cases, hence the use of normal salt, etc. One must remember also to use quartz or corex slips if the specimens are to be compared under ultra-violet radiation.

§ **231. Example of isotropic substances.** — For an isotropic object put an ordinary glass slide under the microscope. Cross the nicols. The field will remain dark.

As an example of isotropic crystals, i.e., those belonging to the cubic system, make a solution of common salt, sodium chlorid (NaCl). Place a drop of the salt solution on a slide that has been tested and found free from strain (§ 222). As the water evaporates crystals will be formed. Place the slide under the microscope, shade the stage well, if not in a darkened room. Cross the nicols. The cubical crystals of salt will remain dark.

§ **232. Examples of anisotropic substances.** — As an example of uniaxial, anisotropic crystals make a fresh preparation of carbonate of lime crystals like that described for pedesis (§ 348), or use a preparation in which the crystals have dried to the slide; use a 4 mm. (40x) objective, shade the object well, remove the analyzer, and focus the crystals; then replace the analyzer. Cross the nicols. In the dark field will be seen multitudes of shining crystals, and if the preparation is a fresh one in water, part of the smaller crystals will alternately flash and disappear. By observing carefully, some of the larger crystals will be found to remain dark with crossed nicols, others will shine continuously. If the crystals are in such a position that the light passes through parallel with the optic axis (§ 232a), the crystals are isotropic like salt crystals and remain dark. If, however, the light traverses them in any other direction, the ray from the polarizer is divided into two constituents vibrating in planes at right angles to each other, and one of these will traverse the analyzer; hence such crystals will appear as if self-luminous in a dark field. The experiment with these crystals from the frog succeeds well with a 2 mm. homogeneous immersion.

As a further illustration of anisotropic objects, mount some cotton fibers in balsam (Ch. XI), also some of the lens-paper (§ 54). These furnish excellent examples of vegetable fibers; striated muscle fibers are also very well adapted for polarizing objects.

§ **232a.** The optic axis of doubly refracting crystals is the axis along which the crystal is not doubly refracting, but isotropic like glass. When there is but one such axis, the crystal is said to be uniaxial; if there are two such axes, the crystal is said to be bi-axial.

The crystals of carbonate of lime from the frog (§ 232) are uniaxial crystals. Borax crystals are bi-axial.

§ **233. Pleochroism, Pleochromatism.** — Polarizing or anisotropic bodies may remove some of the wave lengths of light traversing them. The wave lengths removed depend upon the plane in which the light passing through the substance is polarized with respect to the optic axis of the crystals. For demonstrating the color changes in pleochroism only one element of the polariscope is used. As it is usually easier to remove the analyzer than the polarizer, the polarizer is the element left in place.

If the substance under examination is uniaxial, but two colors are shown, and it is called dichroic.

If the substance is biaxial, then three colors may appear, and the substance is called trichroic.

An easily prepared dichroic substance is hæmin (§ 233a). Find a large hæmin crystal in the preparation and place it at the intersection of the cross hairs of the ocular. Note the position of the stage, then rotate it, and note the changes that take place in a complete revolution, also note the number of degrees of rotation required to make the changes. With the hæmin the crystal will be dark twice in the revolution; and twice it will appear light.

Crystals of acetate of copper show strikingly the dichroic change, the crystals being a part of the time greenish, and a part of the time bluish.

A striking and instructive demonstration to show the difference between pleochromatic and non-pleochromatic objects may be made by adding part of a cotton fiber to the hæmin or the copper when the specimen is prepared. The cotton will not change in color during the entire revolution of the stage. If now the analyzer is added the cotton fiber will be seen to polarize brilliantly.

§ **233a.** Hæmin, hydrochlorate of hæmatin ($C_{34} H_{35} N_4 - N_4 FeO_5 - HCl$) is easily obtained from dried blood. The blood may be fresh or old, it serves, therefore to differentiate the red or brown stains of blood from other reddish brown stains. Hæmin is easily prepared by placing a drop of fresh blood on the middle of a glass slip and spreading it about in a small area. When dry, a crystal or two of common salt (sodium chlorid NaCl) is added and a cover-glass put over the mixture. If old blood is used it should be powdered and several crystals of salt added.

With a pipette add enough glacial acetic acid at the edge of the cover-glass to fill the space under the cover and immerse the blood. Heat in some way till the acid steams well. It is better not to boil the liquid. Examine under the microscope. If successful the brownish red, rhombic crystals of hæmin will be present in abundance. If not add more acetic acid and heat again. When plenty of crystals are present, remove the cover-glass and let the preparation dry. There should be plenty of crystals on the cover-glass as well as on the slide. When thoroughly dry, add a drop of Canada balsam and mount (§ 534).

Hæmin is much used for the detection of blood in medico-legal cases. It can be obtained from stains no matter how old. Hæmin is dichroic (§ 233) and anisotropic (§ 232), but non-fluorescent.

§ **234. Production of colors.** — Many polarizing substances show the most gorgeous coloration under the polarizing microscope. A striking example is white human hair. Short pieces mounted in balsam or other highly refractive medium make a wonderful preparation. As the pieces are at various angles with the plane of polarization some are almost sure to glow with great beauty. By rotating the stage or rotating the specimen by hand different pieces will be in the right position to give the best effects.

A crystalline substance, sulphonal $(CH_3)_2C(SO_2C_2H_5)_2$, is excellent for the dark cross and for the most brilliant coloration. To prepare a specimen, put a large drop of Canada balsam in the middle of a slip and warm it till it is quite liquid. Then add about a third as much dry sulphonal. Heat till the sulphonal melts. Stir with a toothpick to distribute the crystals evenly, and then warm a cover-glass and press it down on the melted mixture. Cool the slide by placing it on a cold body or by putting some alcohol on the back. If the specimen is successful, and it usually is, it will show a great variety. By rotating the stage or the analyzer the black crosses will also rotate and the colors will change to their complements.

COLLATERAL READING FOR POLARIZED LIGHT

BECK, CONRAD. — The Microscope, 1938 ed.
CHAMOT, AND CHAMOT AND MASON. — Chemical Microscopy.
CLARK, C. H. — Practical methods in microscopy (gives sulphonal experiment).
HARDY AND PERRIN. — Principles of Optics.
QUEKETT, JOHN. — Practical treatise on the use of the microscope.
SCHMIDT, W. J. — Die Bausteine des Tierkörpers in polarisiertem Lichte.

SOME OPTICAL PRINCIPLES IN MICROSCOPY

§ **235. Optical facts of prime importance for the microscope.** — In considering the optics of the microscope six fundamental facts concerning light must be kept constantly in mind, for all of them are involved to a greater or less degree in every microscopic observation:

(1) Light is composed of radiation which for visual purposes consists of waves from λ0.4μ to λ0.7μ in length.

(2) Light in a uniform medium extends in straight lines.

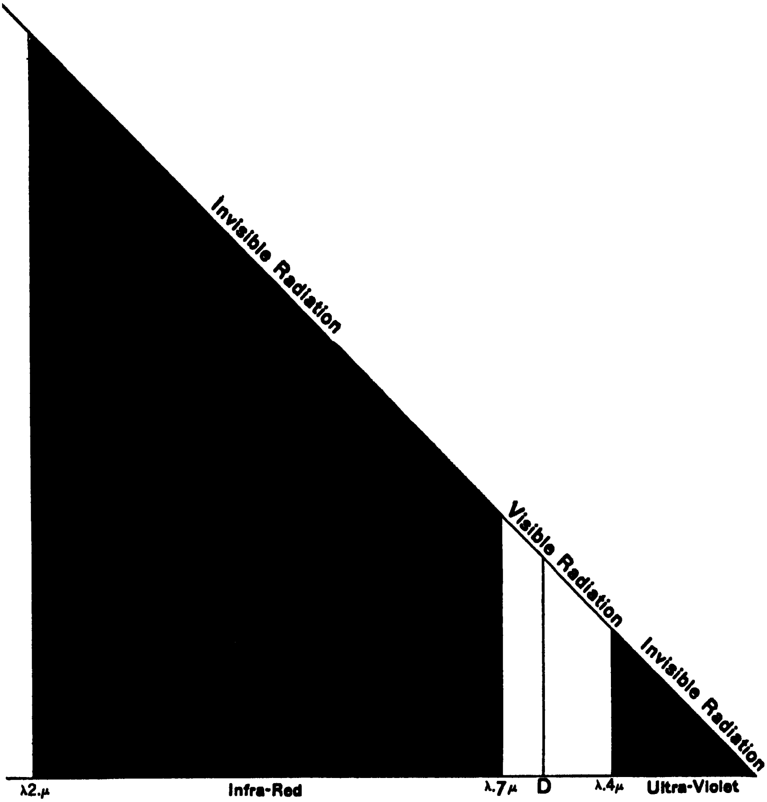

FIG. 93. DIAGRAM OF THE NORMAL SPECTRUM TO SHOW VISIBLE AND INVISIBLE RADIATION.

The spectrum in this diagram is magnified 50,000 times vertically and horizontally. The visible part of the spectrum extends only from about λ.7μ to λ.4μ. *D* represents the dark sodium lines in the solar spectrum. Incandescent sodium in a lamp flame shows a bright line at this level.

(3) Light may be reflected.

(4) Light is refracted in passing from one medium to another of different density.

(5) Light may be dispersed or separated into colored bands from the fact that rays of different wave length are differently bent (figs. 120, 2).

(6) Light may be diffracted, it i.e., bends around small obstacles.

(7) Light may be polarized.

Stated in briefest terms light exhibits the properties of:

(1) Wave motion; (2) Rectilinear propagation; (3) Reflection; (4) Refraction; (5) Dispersion; (6) Diffraction; (7) Polarization.

§ **236. Wave motion.** — From a body like the sun, the electric arc and other sources of energy, radiation is given off which in most respects acts as if it consisted of transverse waves, i.e., waves at right angles to the direction of propagation. The radiation which is visible forms but a very small segment of the total radiation. In fig. 93 the visible radiation is shown between wave lengths $\lambda 0.4\mu$ and $\lambda 0.7\mu$, measured in air or in a vacuum. Shorter waves are called ultra-violet, and longer waves infra-red. The infra-red waves are shown only up to a length of 2μ, although many of much greater length exist.

In the ether of space the different visible waves move with equal velocity, but in the various transparent bodies on the earth, the velocity depends upon the wave length — the shorter the wave, the slower the motion (§ 245).

§ **237. Light moves in straight lines.** — In a uniform medium light moves in straight lines. Any body in which light can tranverse freely is said to be transparent. If light meets a body which it cannot penetrate, it is either reflected (§ 238) or absorbed; if absorbed, it is changed to some other form of energy, usually heat.

§ **238. Reflection.** — If light meets the surface of a body of different refractive index from the medium which it is already traversing the light will be changed in its course.

If the surface is smooth and the light is reflected, the incident and the reflected rays will be in the same plane and will make equal angles on opposite sides of a normal erected at the point of reflection (fig. 94). The eye can see the light only when in the path of the ray, or when light is deflected from the ray by dust, etc. (§ 173).

If the surface is irregular, the reflection will also be irregular and the light will be reflected from the point of incidence in the form of a hemisphere (fig. 95), hence light would reach the eye from any point in the hemisphere.

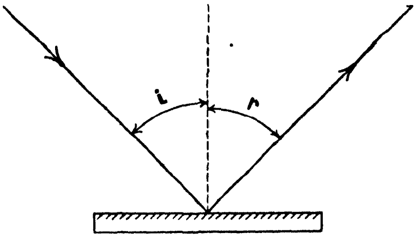

Fig. 94. Regular or Mirror Reflection.

(From Optic Projection).

The angle of incidence *i*, is equal to the angle of reflection *r*; and the incident and reflected ray are in a plane perpendicular to the reflecting surface.

§ 239. Refraction. — As ordinarily considered, this is the change in direction which light undergoes when passing obliquely from one transparent medium into another (figs. 96–98).

A broader statement covering all the phenomena, whether the ray passes obliquely or normally from one medium to another, is this: Refraction is the change in velocity of the waves of light in passing from one transparent medium into another.

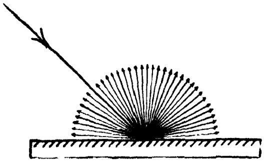

Fig. 95. Irregular or Diffuse Reflection.

(From Optic Projection).

A ray of light meeting a rough surface, like a piece of white paper, is scattered almost equally in all directions, making a hemisphere of light.

§ 240. Law of refraction. — The amount of bending depends upon two factors, — the relative density of the two media and the obliquity of the incident light. The greater the obliquity of the incident ray, and the greater the difference in density, the greater will be the refraction. The precise law governing the course and relation of the ray in the two media is known as the sine law of Snell and Descartes. It is expressed thus: $\frac{\sin i}{\sin r}$ = index of refraction. That is, the sine of the angle of the incident ray with the normal to the dividing surface divided by the sine of the angle of the refracted ray with its normal, gives the relative direction of the ray in the two media, i.e., the index of refraction. For example, in fig. 96, showing the passage of light to water, the ray being at 60° with the normal in air, and 40° 38′ in water, the real relationship in this and in all other cases is not the relative size of the two angles, but the sines of the angles, thus: $\frac{\sin i \text{ or } 0.86603}{\sin r \text{ or } 0.65115} = 1.33$. That is, the sine of the angle in air is 1.33 times the sine of the angle in water; and this would hold true for any other pair of sines, so that the law is universal for the wave length of light giving this index of refraction.

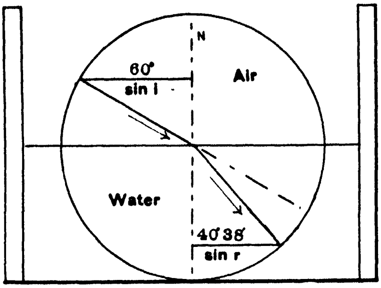

FIG. 96. REFRACTION OF LIGHT IN PASSING FROM AIR TO WATER.

N Normal at the point of refraction.
$\frac{\sin i \text{ In this example } \sin 60° \text{ or } 0.86603}{\sin r \text{ In this case } \sin 40° 38' \text{ or } 0.65115}$ = 1.33, average index of refraction for air and water.

The sine and corresponding angle are always greater in the rarer medium and consequently less in the denser medium. It follows from this that when the ray passes from a rarer to a denser medium and the angle is made less, the ray must bend *toward* the normal. Conversely, in passing from a denser to a rarer medium where the angle is greater, the ray must bend *from* the normal. This is a general law (see figs. 98, 100–102).

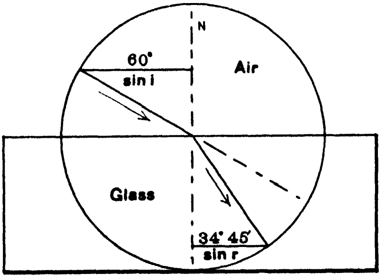

FIG. 97. REFRACTION OF LIGHT IN PASSING FROM AIR TO GLASS.

N Normal at the point of refraction.
$\frac{\sin i \text{ In this example } \sin 60° \text{ or } 0.86603}{\sin r \text{ In this example } \sin 34° 45' \text{ or } 0.57000}$ = 1.52, average index of refraction for air and glass.

§ **241. Absolute index of refraction.** — This is the index of refraction obtained when the incident ray passes from a vacuum into a given medium. As the index of the vacuum is taken as unity, the absolute index of any substance is always greater than unity. For many purposes, as for the object of this book, air is treated as if it were a vacuum, and its index is called unity, but in reality the index of refraction of air is about 3 ten-thousandths greater than unity. Whenever the refractive index of a substance is given, the absolute index is meant unless otherwise stated. For example, when the index of refraction of water is said to be 1.33, and of crown glass 1.52, etc., these figures represent the absolute index, and the incident ray is supposed to be in a vacuum.

§ **242. Relative index of refraction.** — This is the index of refraction between two contiguous media, as, for example, between glass and diamond, water and glass, etc. It is obtained by dividing the absolute index of refraction of the substance containing the refracted ray, by the absolute index of the substance transmitting the incident ray. For example, the relative index from water to glass is 1.52 divided by 1.33. If the light passed from glass to water, it would be 1.33 divided by 1.52.

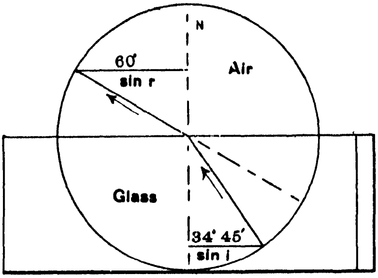

FIG. 98. REFRACTION OF LIGHT IN PASSING FROM GLASS TO AIR.

N Normal to the refracting surface.

$$\frac{\sin i \text{ In this case } \sin 34^\circ 45' \text{ or } 0.57000}{\sin r \text{ In this case } \sin 60^\circ \text{ or } 0.86603} = \frac{1}{1.52}$$

If figs. 97 and 98 are compared it will be seen that the ray of light follows exactly the same path in leaving the denser medium that it took on entering it.

By a study of the figures showing refraction, it will be seen that the greater the refraction the less the angle and consequently the less the sine of the angle, and as the refraction between two media is the ratio of the sines of the angles of incidence and refraction $\left(\frac{\sin i}{\sin r}\right)$, it will be seen that whenever the sine of the angle of refraction is increased by being in a less refractive medium, the index of refraction will show a corresponding *decrease* and *vice versa. That is,* *the ratio of the sines of the angles of incidence and refraction of any two contiguous substances is inversely as the refractive indices of those substances.* The formula is:

$$\left(\frac{\text{Sine of angle of incident ray}}{\text{Sine of angle of refracted ray}}\right) = \left(\frac{\text{Index of refraction of refracting medium}}{\text{Index of refraction of incident medium}}\right)$$

Abbreviated $\left(\frac{\sin i}{\sin r}\right) = \left(\frac{\text{index } r}{\text{index } i}\right)$. By means of this general formula one can solve any problem in refraction whenever three factors of the problem are known. The universality of the law may be illustrated by the following cases:

(A) Light incident in a vacuum or in air, and entering some denser medium, as water, glass, diamond, etc.

$$\left(\frac{\text{Sine of angle made by the ray in air}}{\text{Sine of angle made by the ray in denser med.}}\right) = \left(\frac{\text{Index of ref. of denser med.}}{\text{Index of ref. of air (1)}}\right)$$

If the dense substance were glass: $\left(\frac{\sin i}{\sin r}\right) = \left(\frac{1.52}{1}\right)$. If the two media were water and glass, the incident light being in water the formula would be: $\left(\frac{\sin i}{\sin r}\right) = \left(\frac{1.52}{1.33}\right)$. If the incident ray were glass and the refracted ray in water: $\left(\frac{\sin i}{\sin r}\right) = \left(\frac{1.33}{1.52}\right)$. And similarily for any two media; and as stated above if any three of the factors are given the fourth may be readily found.

§ 243. Critical angle and total reflection. — In order to understand the Wollaston camera lucida (fig. 168) and other totally reflecting apparatus, it is necessary briefly to consider the critical angle.

The *critical angle* is the greatest angle that a ray of light in the denser of two contiguous media can make with the normal and still emerge into the less refractive medium. On emerging, it will form an angle of 90° with the normal, and if the surface is flat the refracted ray will be parallel with the surface separating the two media.

Total Reflection. — In case the incident ray in the *denser* medium is at an angle with the normal greater than the *critical angle*, it will be *totally reflected* at the surface of the denser medium, that surface acting as a perfect mirror. By consulting the figures it will be seen that there is no such thing as a critical angle and total reflection in the *rarer* of two contiguous media.

To find the critical angle in the denser of two contiguous media: — Make the angle of refraction (i.e., the angle in the rarer of the two media) 90° and solve the general equation: $\left(\frac{\sin i}{\sin r}\right) = \left(\frac{\text{index } r}{\text{index } i}\right)$.

(1) Critical angle of water and air: sin r (90°) is 1, index of water 1.33, whence $\left(\frac{\sin i}{1}\right) = \left(\frac{1}{1.33}\right)$ or $\sin i = 0.751 +$. This is the sine of 48° 45′, and whenever the ray in the water is at an angle of more than 48° 45′ it will not emerge into the air, but be totally reflected back into the water.

(2) Critical angle of glass and air: sin r (90°) is 1. Index for glass is 1.52, whence $\left(\frac{\sin i}{1}\right) = \left(\frac{1}{1.52}\right) = \sin\ 0.65789$, which is the sine of 41° +. Light having a greater angle in glass than 41° is internally reflected as from a mirror (fig. 94).

(3) Critical angle of glass covered with water.

$$\left(\frac{\sin i}{\sin r\ (\sin 90^\circ = 1)}\right) = \left(\frac{\text{index water } (1.33)}{\text{index glass } (1.52)}\right) \text{ or } \left(\frac{\sin i}{1}\right) \left(\frac{1.33}{1.52}\right).$$

whence $\sin i = .875$, sine of critical angle in glass covered with water. The corresponding angle is approximately 61°.

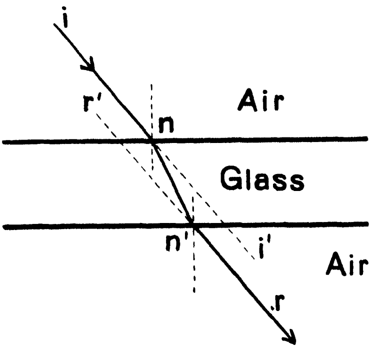

FIG. 99. DISPLACEMENT OF A RAY OF LIGHT IN TRAVERSING AN OBJECT WITH PLANE FACES.

This figure is to show that while there is no angular deviation of a ray of light in traversing a dense medium with plane faces, there is displacement; but the emerging ray (r) is parallel with the entering ray (i).

Air Glass The two media through which the ray is traveling.

i n Incident ray and normal at the point of entrance into the glass.

i′ Incident ray continued by dotted lines to show the path which would have been followed if no glass had intervened.

n′r Normal and refracted ray on emergence from the glass to the air again.

r′ Path of the refracted ray traced backward.

The last shows the advantage of water immersion when a large angle of light is desired. With homogeneous immersion there would be no critical angle for the glass.

§ **243a. Critical angle.** — As defined by some physicists the critical angle is the least angle at which light undergoes total internal reflection at the surface of the denser medium.

I have followed the more common definition which makes it the greatest angle at which a ray can emerge into the rarer medium; the emerging angle will then be 90° and its sine 1.000.

§ 244. Table of refractive indices n_D. (From Chamot.)

(Temperature 20 to 22 C.)

Index of Refraction	Name of Substance	Approximate Boiling Point °C.	Approximate Density
1.32	Methyl alcohol	66	0 79
1.36	Ethyl ether	35	0 71
1.37	Ethyl alcohol	78	0 79
1.46	Cajeput oil	174	0.92
1.44	Chloroform	61	1 48
1.47	Glycerine	290	1 61
1.47	Turpentine	155	0 86
1.48	Castor oil	.	0 96
1.49	Xylene	136	0 86
1.49	Benzene	80	0 88
1.50	Clove oil	...	1.05
1.51	Cedar Wood oil	...	0 98
1.57	Orthotoluidine	197	1 00
1.625	Carbon bisulphide	46	1 29
1.52 ±	Canada balsam	...	...
1.52–1.59	Glass	...	....
1.544–1.553	Quartz	...	...

§ **244a.** Index of refraction and wave length. — As the shorter waves of the blue end of the spectrum are more bent than the long waves of the red end in it indicates that the index of refraction is greater for the blue end than for the red end. Unless otherwise indicated, the index of refraction (n) is that of the D line in the spectrum, and if written out entire the index would read $\frac{n_D i}{n_D r}$

At the H line it would be expressed thus $\frac{n_H i}{n_H r}$

Specific cases from Watson's Physics:

n_D for water 1.334	n_H for water............1.34
n_D for flint glass 1.584	n_H for flint glass 1.614

It is further to be noted that there is not perfect regularity in the increase or decrease of the index of refraction according to the wave length of the light. The exact index in each case must be determined experimentally. As will be seen later, this irregularity makes it possible to construct achromatic instruments (§ 258).

§ 245. The sine law and the velocity of light in different media. — In the ether of space all wave lengths of light move with equal velocity, but on the earth the velocity depends on the wave length. While all wave lengths are retarded by shortening the waves, the shorter the original wave the greater the retardation. As the refraction of the light is one of the phenomena of this retardation, it

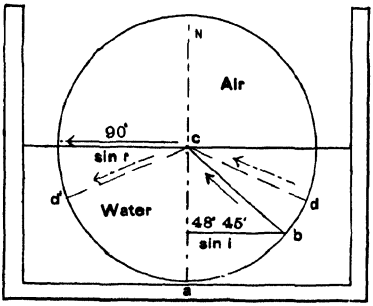

FIG. 100.

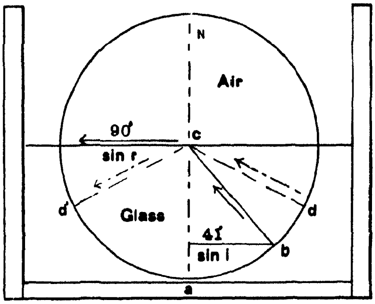

FIG. 101.

FIG. 100. CRITICAL ANGLE FOR LIGHT PASSING FROM WATER TO AIR, THE ANGLE IN AIR BEING 90°.

N Normal to the refracting surface.

$\dfrac{\mathit{sin}\ i \text{ In this case } \mathit{sin}\ 48^\circ\ 45' \text{ or } 0.7519}{\mathit{sin}\ r \text{ In this case } \mathit{sin}\ 90^\circ \text{ or } 1.0000} = \dfrac{1}{1.33}$, in accordance with the general formula: $\dfrac{\mathit{sin}\ i}{\mathit{sin}\ r} = \dfrac{\text{index } r}{\text{index } i}$

b Light ray at the critical angle and emerging into the air parallel with the surface of the water.

d d' Ray of light at an angle greater than the critical one and being internally reflected back into the water; the angle of incidence and reflection being equal (fig. 94).

FIG. 101. CRITICAL ANGLE FOR LIGHT PASSING FROM GLASS TO AIR, THE ANGLE IN AIR BEING 90°.

N Normal to the refracting surface.

$\dfrac{\mathit{sin}\ i \text{ In this case } \mathit{sin}\ 41^\circ + \text{ or } 0.65789}{\mathit{sin}\ r \text{ In this case } \mathit{sin}\ 90^\circ \text{ or } 1.0000} = \dfrac{1}{1.52}$, in accordance with the general formula: $\dfrac{\mathit{sin}\ i}{\mathit{sin}\ r} = \dfrac{\text{index } r}{\mathit{index}\ i}$

b Light ray at the critical angle and emerging into the air parallel with the surface of the glass.

d d' Ray of light at an angle greater than the critical angle and being reflected back into the glass, the angle of incidence and reflection being equal (Fig. 94).

follows that the shorter the wave the greater the bending. This is shown by the action of the prism (fig. 120, 2), in which the blue is more deviated than the red.

The retardation of any given wave length (i.e., the relative shortening of the waves) follows the sine law in passing from one transparent substance to another. For example, in passing from the ether to water, the speed in water would be represented by: $\frac{\sin i}{\sin r}$ or 1.334 for waves at the D Fraunhofer line. (Nichols, Southall, Watson.) This means that if the speed in the ether were 1, in water for this wave length the velocity would be $\frac{1}{1.334}$. In terms of the angle of the light, if the sine of the angle in the ether is 1, the sine of the angle of this wave length in water would be $\frac{1}{1.334}$.

For crown glass the waves opposite the fixed line *B*, if possessed of a speed of 1 in the ether, would have a speed in the glass of $\frac{1}{1.531}$. Opposite the *H* line, with the shorter waves, the speed would be $\frac{1}{1.551}$ in crown glass.

That is, then, just as in refraction (§§ 239–240), if the velocity in one medium and the index of refraction of the two media are known the velocity in the second medium can be determined; and in general, knowing any three factors, the fourth can be determined.

While for the discussion of lenses the narrower view of refraction may suffice, for optical instruments generally it is of fundamental importance to realize that there is just as much effect on light waves striking the surface of the refracting body perpendicularly as obliquely. In one case, that of the oblique meeting, the ray is bent because of the shortening of the waves in passing from a rarer to a denser medium. If the waves meet the denser substance normally to its surface, the ray will not be bent, but the shortening of the waves will be the same, leading to an optical shortening of the path of the ray. This is of prime value when designing optical apparatus where two optical paths must be made equal, although the actual distance in millimeters may be unequal. The binocular microscope is a striking example (figs. 29–31). The shortening of the path is also very strikingly illustrated by the cover-glass (figs. 52 *B–C*, §§ 105–106).

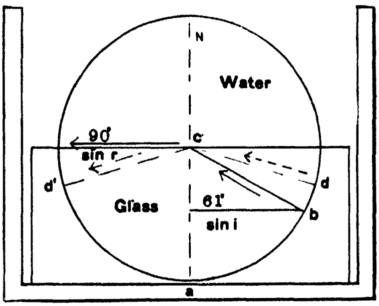

FIG. 102. CRITICAL ANGLE FOR LIGHT PASSING FROM GLASS TO WATER, THE ANGLE IN THE WATER BEING 90°.

N Normal to the refracting surface.

$\frac{\sin i}{\sin r}$ $\frac{\text{In this case } \sin 61^\circ + \text{ or } 0.8750}{\text{In this case } \sin 90^\circ \text{ or } 1.0000} = \frac{1.33}{1.52}$ in accordance with the general formula $\frac{\sin i}{\sin r} = \frac{\text{index } r}{\text{index } i}$

b Light ray at the critical angle and emerging into the water at an angle of 90° from the normal.

d d' Ray of light at an angle greater than the critical angle and hence reflected back into the glass, the angle of incidence and reflection being equal.

§ **246. Dispersion by glass, etc.** — This is the separation of the waves of white light into groups according to their length; and these different groups appear of different colors to the normal eye. When white light is dispersed by a glass prism there results a spectrum or rainbow with the red at one extremity and the blue-violet at the other. As this dispersion into colors is made possible by the different refrangibility of the different wave lengths, one would expect that the amount of bending would be in exact proportion to the wave length. This is true if one uses a grating and produces a normal spectrum (fig. 121). When a prism is employed to produce the dispersion, the refraction is not in exact relation to the wave length. In general, the blue end of the spectrum is expanded and the red end contracted. Different kinds of glass and transparent minerals (quartz, fluorite, etc.) refract differently. This makes achromatism possible. As pointed out by Newton, if the refraction were in exact proportion to the wave length, as with gratings, whenever dispersion is overcome, the general refraction would also be overcome and no achromatic combinations of lenses would be possible.

§ **247. Diffraction.** — This is the bending of light past the edge of objects. Instead of the light all going in a straight line beyond an object, especially a narrow strip, some of it extends as if split off from the main beam at the edge of the obstruction. These diffracted beams may give rise to independent or so-called spurious images. With low powers the diffracted light does not cause complications, but with high powers the diffraction fringes and diffraction disc may produce effects very difficult of interpretation. (See § 270 where there is a discussion of the part played by diffracted light in microscopic images).

LENSES AND IMAGES

§ **248. Lenses.** — A lens is a transparent body having one or both of its opposite sides curved. The curves are most frequently spherical, and may be either convex or concave. If both the surfaces are curved, the lens may be considered as composed of segments of two spheres. These spheres are of like radius if the surfaces are similarly curved, and of unlike radius if the surfaces are unlike. While a lens with one plane face may be considered a segment of a single sphere, optically it is better to consider two spheres, the curved surface from a sphere of finite, and the plane face from a sphere of infinite radius (fig. 107, 3, 6).

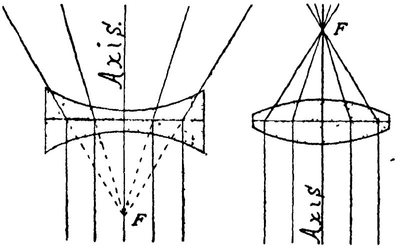

FIG. 103–104. A CONCAVE LENS SHOWING THE PRINCIPAL, VIRTUAL FOCUS; AND CONVEX LENS SHOWING THE REAL PRINCIPAL FOCUS (F F).

§ **249. Images formed by lenses.** — As light entering a dense transparent body obliquely is bent toward the normal at the point of entrance, it follows that if the lens has convex faces, the light rays will be made more convergent; if it has concave faces, the light rays will be rendered more divergent (figs. 103–104). From the change in the direction of the rays on entering and on leaving a lens, it is possible to form images of objects by means of lenses (figs. 105–106).

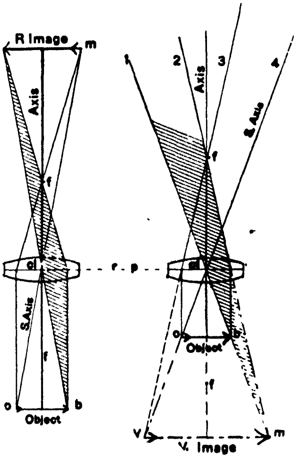

FIG. 105. TO SHOW THE FORMATION OF A REAL AND OF A VIRTUAL IMAGE BY A CONVEX LENS. (COMPARE FIG. 11–12).

The size of the image depends upon its relative distance from the center of the lens. If it is farther from the center than the object, it will be larger than the object, but if nearer, it will be smaller (fig. 152).

§ 250. Forms and principal features of spherical lenses. — As shown in fig. 107, lenses may be convex on both faces, or convex on one face and plane or concave on the other. Lenses may also be concave on both faces or concave on one face and plane or convex on the other.

If lenses are thick in the middle and thin on the edge, they make the rays of light entering them more convergent. On the other hand, if they are thin in the middle and thick on the edge, they make the light rays entering them more divergent. In a word, then, thin edge lenses are called convergent, and thick edge ones, divergent lenses. This follows inevitably from the rule that, on entering a denser medium, any oblique ray of light is bent toward the normal, and on leaving it for a rarer medium, it is bent from the normal (§ 240).

§ 251. Principal features of spherical lenses. — (1) Principal axis. This is the straight line passing through the lens and joining the centers of the two spheres supposedly contributing to the formation of the lens (fig. 107, 4*c c'*).

(2) Optic center. This is the point in a lens or near it through which light rays pass without angular deviation. That is, the ray passing through the center of the lens continues in a line parallel to

the original direction as it does in traversing a piece of plane glass (fig. 99).

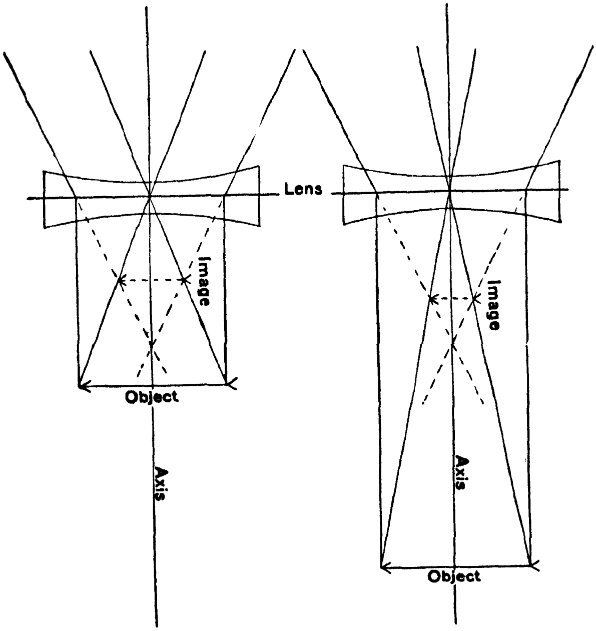

FIG. 106. TO SHOW THE FORMATION OF A REDUCED VIRTUAL IMAGE BY A CONCAVE LENS, AND THAT THE IMAGE IS LARGER THE NEARER THE OBJECT IS TO THE PRINCIPAL (VIRTUAL) FOCUS. (COMPARE FIG. 154–155).

As shown in the diagrams (fig. 107), the optic center is found by drawing parallel radii from the two curved surfaces, or from the curved and plane surface, and joining the ends of the radii. The center of the lens is at the point where a line connecting the ends of the radii crosses the principal axis (fig. 107 *cl.*) The reason light rays traversing the optic center have no angular deviation is evident, for the radii are perpendicular to the surface of the lens, and the tangent plane perpendicular to the radius is tangent to the sphere at the end of the radius. As the tangents of two parallel

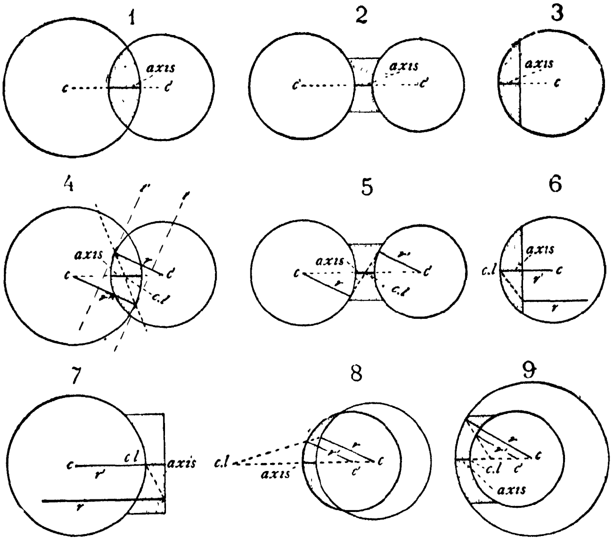

FIG. 107. SPHERICAL LENSES WITH THEIR FORMS AND PRINCIPAL FEATURES.

(1) Double convex lens showing the two spheres from which it was derived. *c-c'* the centers of the two spheres with the principal axis of the lens on the line joining the centers.

(2) Double concave lens and the two spheres from which it was derived. *c-c'* centers of the spheres and axis of the lens.

(3) Plano-convex lens with the sphere from which it was derived. In this case the axis is on the radius dividing the lens into two equal parts.

(4) Double convex lens showing the two spheres from which it was derived; *r r'* parallel radii; *t t'* tangents at the ends of the radii; *c c'* centers of the two spheres from which the lens was derived. The line connecting the centers is the optic axis. The center of the lens (cl) is on this axis.

(5) Double concave lens showing the same features as in (4).

(6) Plano-convex lens showing the same as in (5). In this case the radius of the curved face is determined as usual, but that of the plane face may be considered infinity, so that any line perpendicular to the plane face is a part of that radius. As shown in the figure the center of the lens must be then at the convex surface of the lens.

(7) Plano-concave lens the parts are practically like (6).

(8) Thin edge or converging meniscus lens with the two spheres from which it was derived. The inner, concave face is from the greater sphere, and the optic center (*cl*) is wholly outside the lens.

(9) Thick edge or diverging meniscus lens. In this case the concave face is from the smaller sphere, and the center of the lens (*cl*) is on the concave side.

radii must themselves be parallel, it follows that a ray of light passing from one tangential point to the other is traversing a body with parallel sides at the point of entrance and exit, and hence it will suffer no angular deviation. The ray may be displaced as in traversing any thick transparent body (fig. 99). With meniscus lenses the optic center (fig. 107, 8, 9) is on an extension of the line joining the centers of curvature, and wholly outside the lens.

(3) Secondary axis. This is any line which passes through the optic center of the lens and is oblique to the principal axis.

(4) Principal focal point. The principal focal point or focus of a lens or of a lens system like an objective, a simple microscope, etc., is the point on the principal axis where rays of light parallel to the principal axis before entering the lens or lens system, cross the principal axis after leaving the lens or objective (figs. 103–104). The focus is also called the burning point. With a concave mirror it is the point on the principal axis where rays parallel with the principal axis before meeting the mirror, cross the principal axis after reflection from the concave surface. This point is situated half-way between the face of the mirror and the center of curvature.

ABERRATION OF LENSES

§ **252. Spherical aberration.** — This is a defect of spherical lenses shown in fig. 108. That is, the parallel ray at the edge crosses the principal axis or comes to a focus nearer the center of the lens than a ray near the axis. If, then, the full aperture is filled, as shown in the figure, with rays parallel with the axis, there will be a series of foci, those of the border rays being nearer the lens than those near the middle of the lens (fig. 108, f1, f2, f3).

§ **253. Correction of spherical aberration.** — It is possible by selecting convex and concave lenses of different material and hence of different refractive power, to overcome the spherical aberration of the convex lens with an equal and opposite aberration in a concave lens without overcoming the converging action of the convex lens. Consequently rays will all come to one focus. Such a lens combination is said to be aplanatic or spherically corrected, If the correction were not quite sufficient so that the border rays still came to a focus slightly nearer the lens than the middle rays, the combination would be *under-corrected.* If the concave lens were too strong, the border rays of the convex lens would come to a focus farther from the lens than the middle rays, and the combination would be said to be *over-corrected.* Sometimes under-correction or over-correction is designed to compensate for parts of the optical apparatus which the rays will meet later, or for aberrations produced before the light reaches the objective. The common and almost universal example is the spherical aberration introduced by the cover-glass over the specimen (fig. 109).

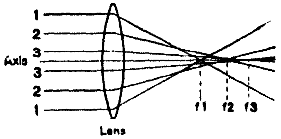

FIG. 108. SPHERICAL ABERRATION IN LENSES.

Axis The principal optic axis.

1 2 3 Ray 1 at the edge comes to a focus at *f 1;* ray 2 at *f*2, and ray 3 at *f*3, that is, the nearer the optic axis, the longer the focus; and the nearer the edge of the lens, the shorter the focus.

§ **254. Cover-glass correction.** — By referring to fig. 109 it will be seen that the effect of the cover-glass is precisely like the spherical aberration due to the unequal refraction of the different zones of a convex lens; that is, the border rays are more bent than those nearer the axis, as the obliquity of the rays is greater (§ 240).

Now to overcome this there must be introduced into the objective an under-correction just sufficient to balance the effect of the cover-glass. If the lenses are fixed in position in the objective it will be evident that one must select a cover-glass which is of the exact thickness to satisfy the correction of the objective. The makers of objectives are now very precise in stating exactly how thick the covers should be for their objectives, and it is the part of wisdom to pay heed to their statements if one hopes to get the best results.

If one's objectives are adjustable (§§ 149–150), it is possible to arrange the combinations so that quite a range of cover-glass thickness or mounting medium thickness can be used and still get the best optical effect by balancing the aberrations (§ 256).

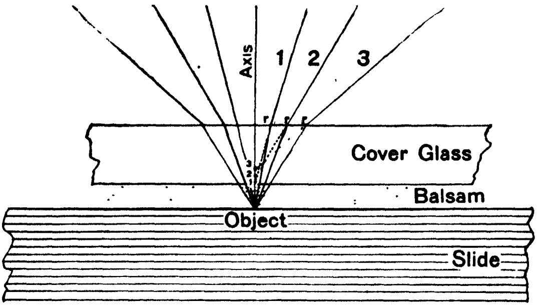

FIG. 109. SPHERICAL ABERRATION INTRODUCED BY THE COVER-GLASS.

Axis The principal optic axis extending through the condenser and up through the object and microscope.

Slide The glass slide on which the object is mounted.

Object The object to be studied; it is mounted on the slide.

Balsam The medium in which the object is mounted. It has practically the same refractive index as the cover.

Cover-glass The thin glass plate over the object.

1 2 3 The light rays extending obliquely upward from the object.

3 2 1 Light rays traced backward to their apparent origin, the most oblique ray (*3*) being most bent, thus rendering its origin apparently highest.

r r r Points of refraction of the three oblique rays.

§ **255. Tube-length.** — The length of the tube on the microscope must be made of the standard for which the objective used was corrected or aberrations will appear.

If the tube is shorter than the objective was corrected for, the effect is the same as thinning the cover-glass. That is, it introduces under-correction. This makes it possible to compensate for too thick a cover by shortening the tube (§§ 150, 256).

When homogeneous immersion liquid is used one does not have to trouble about the exact thickness, but care must be taken not to use so thick a cover that the free working distance will be too short (§ 101).

By consulting the catalogues of microscope manufacturers one can find for what tube-length and thickness of cover-glass their unadjustable objectives are corrected. For example, in the last editions of the catalogues of the Bausch & Lomb Optical Company of Rochester, and of the Spencer Lens Company of Buffalo, it is stated that the tube-length is 160 millimeters and, as shown in the accompanying figure (fig. 110), it includes the parts from the upper end of the draw-tube to the nut into which the objective is screwed.

The cover-glass thickness is given as 0.18 millimeter, and the user is warned that for the higher powers a variation in thickness from this standard of 0.03 or 0.04 mm. would deteriorate markedly the perfection of the image. The statement is furthermore made that with the homogeneous immersions no harm would result from varying thickness of cover-glass, but on the other hand great care must be exercised there to use the correct tube-length or aberrations will be introduced.

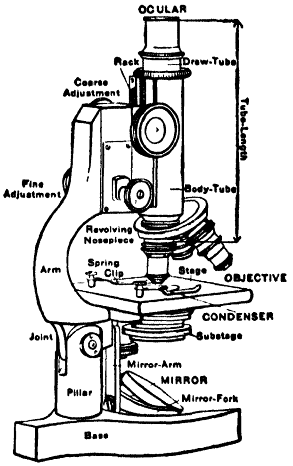

FIG. 110. THE MICROSCOPE SHOWING TUBE-LENGTH

§ 256. Table showing cause of spherical aberration in the microscope and means of correction. —

Under-correction produced by:	Over-correction produced by:
1. Too weak a concave element in the objective.	1a. Too strong a concave element in the objective.
2. Too close an approximation of the lenses of the objective.	2a. Too great a separation of the lenses of the objective.
3. Too short a tube, that is, the ocular and objective are too close together.	3a. Too long a tube, that is, the ocular and objective are too far apart.
4. Use of too thin a cover-glass.	4a. Use of too thick a cover-glass.

Any defect can be neutralized by applying the right amount of what would produce the opposite condition. For example, the over-correction produced by too thick a cover-glass can be corrected by: (4) using a thinner cover-glass; (3) shortening the tube; (2) putting the lenses of the objective closer together; (1) using a weaker concave element in the objective.

If there is under-correction from too short a tube it can be neutralized by: (3a) lengthening the tube; (4a) using a thicker cover-glass; (2a) separation of the lenses of the objective; (1a) using a stronger concave element in the objective. And similarly with under-correction or over-correction from any cause; opposites neutralize.

§ **257. Chromatic aberration.** — Spherical aberration which has just been discussed is present in lenses even when the light is of one wave length; chromatic aberration, on the other hand, appears in addition when composite light traverses a lens. That is, every wave length of necessity is differently refracted; the shortest waves most, the longest waves least. If then a single beam of white light traverses a lens, the different wave lengths will be refracted differently and the blue-violet waves made to cross the axis first, the red waves last. There will be then a series of colored foci extending along the axis, as shown in fig. 111. Every simple lens, then, whose aperture is filled with composite light, will show both spherical and chromatic aberration, and the greater the aperture and the shorter the focus the more pronounced will be both forms of aberration. In order that perfect images may be produced, both aberrations must be eliminated.

Fortunately the visible spectrum does not include a greater range of wave lengths (fig. 93), and if it were markedly less, the optician would find his task greatly lightened. As shown in fig. 210, the brightest region of the spectrum to the eye is really limited, and the old opticians made good instruments for visual purposes by overcoming the aberrations in large part in this very limited region; but with the requirements of photography and for the most complete visual study of the phenomena and objects of nature by means of optical instruments, greater and still greater demands were made for optical instruments including at least the whole visible spectrum, and for some purposes extending into the infra-red and the ultra-violet.

§ **258. Correction of the aberrations of lenses.** — From the very law of refraction bound up with the different wave lengths of visible light it would seem impossible to obtain the refraction necessary to produce images (figs. 105–111) without at the same time dividing the light up into its colors. If the refraction of each wave length were in exact proportion to its length, as with a diffraction grating, it would be impossible to produce achromatic images. Newton thought the refraction was always as with a grating, and he explained the satisfactory images produced by lenses on the ground that the narrow part of the spectrum most brilliant to the eye overwhelmed the dimmer parts so that the colored images on both sides of the visual image were ignored.

If one compares, however, the spectrum produced by the diffraction grating (fig. 121) with that produced by a glass prism (fig. 122), it will be seen that the refraction of the different wave lengths (dispersion) differs very markedly in the two cases, although the total length of the spectrum is the same in both.

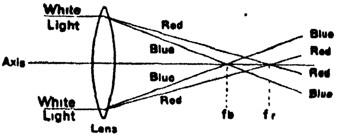

Fig. 111. Chromatic Aberration with Composite Light.

White light A beam of white light composed of all the colors meeting a lens and the different wave lengths being differently refracted breaks the composite light up into its constituent colors.

Red Blue The long waved red light is less refracted than the shorter waved blue light. After crossing at the foci the blue light is on the outside of the diverging cone.

fb, fr The focus of the blue light (*fb*) is nearer the lens than the focus of the red light (*fr*).

Axis The optic axis of the lens.

The dispersion or separation into colors differs with different transparent substances, and is not in proportion to the mean refraction.

The red is much contracted and the blue expanded with the glass prism. One can then have what might be called a mean refraction with the glass prism, the refraction of the individual groups of wave lengths not being in proportion to the lengths. Now it is from this irregularity of the refraction in different parts of the spectrum, and because the irregularity differs with different transparent substances, that it is possible to have the refraction necessary to produce images without having the light dispersed into colors at the same time. This is shown in fig. 112, 2, where a smaller prism of flint glass produces the same amount of dispersion as a larger prism of crown glass. If these prisms are with their edges opposite, the spectrum produced by the flint glass will be brought together by the crown glass and white light will result; but as the mean refraction of the larger crown glass prism is greater than that of the flint glass prism, the ray of white light will not extend parallel with the original direction, but be bent toward the base of the crown glass prism. As a lens may be considered an infinite number of prisms combined, it becomes intelligible from this how it is possible to produce colorless images by combining flint-glass concave and crown glass convex lenses; or other pairs of lenses where the dispersion and refraction give comparable results.

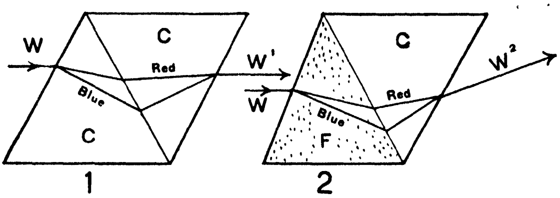

FIG. 112. ACHROMATISM BY COMBINING DIFFERENT KINDS OF GLASS.

(1) White light (*W*) traversing two equal crown glass (*CC*) prisms with their bases opposite. The dispersion into a spectrum by the first prism is overcome by the second prism and the light is recombined into a white beam (W^1), which is displaced as if it had traversed a piece of plane glass.

Red Blue The red and the blue edges of the spectrum. The blue is more refracted than the red.

(2) White light (*W*) traversing a flint glass prism (*F*) and being dispersed into the spectral colors. The spectrum formed by the flint prism is recombined by the crown glass prism (*C*), but the emerging ray of white light (W^2) is refracted markedly toward the base of the crown glass prism, showing the possibility of an achromatic image. The arrows show the direction in which the light is extending.

In making the color corrections for the lenses, the spherical corrections were also made; the extent of both corrections attained up to the present is discussed below.

§ 259. Corrections in Achromatic and Apochromatic objectives. — (1) Spherical aberration. In achromatic objectives the spherical aberration is corrected for one color only, in apochromatic objectives for two colors. (2) Chromatic aberration. In achromatic objectives correction is made for two colors; in apochromats for three colors.

In the apochromats it was found impossible to make the high corrections necessary even with all the new glasses made available by the Jena glass works; but with the new forms of glass and a natural mineral, fluorspar, fluorite, calcium fluoride, with its very low index of refraction and small dispersion, it was found possible to make the fundamental advance in microscope objectives represented by the apochromatic objectives.

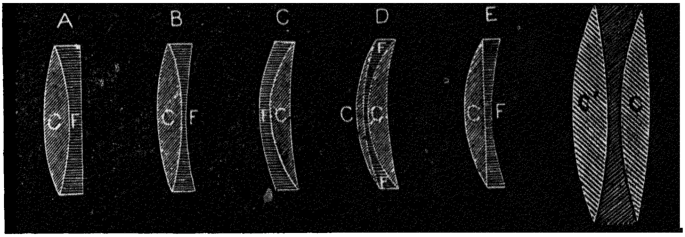

Fig. 113. Achromatic Combinations of Crown and Flint Glass Lenses.

(From Lewis Wright's Optical Projection).

C C C C C C Thin edge or converging crown glass lenses.
F F F F F F Thick edge or diverging flint glass lenses. The flint glass overcomes the dispersion without overcoming the mean refraction, hence all these combinations are converging.

The possibility of bringing three colors to one focus makes the apochromatic objectives especially valuable for photography. The visual and actinic foci are coincident, and if the apparatus is well constructed, there is never any difficulty in getting sharp pictures, for the photographic image is sharpest when it appears sharpest to the normal eye.

§ 260. Compensation oculars. — As the front lens of objectives of high power (figs. 20, 21) is not a combination but a single lens, aberrations are inevitably introduced which must be eliminated by a subsequent part of the optical train. The most striking and troublesome defect is the so-called difference of chromatic magnification, that is, the differently colored constituent images forming the final image are of different magnitudes, the blue one being larger than the red one. This defect is more easily corrected in the ocular than in the subsequent combinations of the objective. The ocular is then constructed to give a red image sufficiently large to bring its magnification up to that of the blue image, and hence the final image as seen by the eye is correct. The low power apochromats could be corrected for this, but for the sake of using the same oculars on all

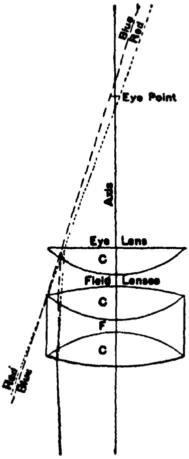

FIG. 114. POSITIVE COMPENSATION OCULAR.

(From Spitta, p. 110).

C F C The field lens is composed of two double convex crown lenses and one double concave, flint glass lens.

C The eyelens is of crown glass, and is separated from the field combination the right distance to give the necessary excess magnification of the red image to make it balance the blue image which was over magnified by the objective.

Red Blue The red and blue rays limiting the image. It is seen here that the rays are not parallel but divergent, as they extend above the ocular. When projected by the eye to the virtual image, the rays cross, throwing the red one to the outside, thus giving a larger image than is given by the blue ray, and the orange haze at the margin of the field when looking through the ocular toward the window or the sky.

§ **259a.** It is interesting to note that the wonderful optical qualities of fluorspar were known to Sir David Brewster, and recommended by him for aid in achromatization (Brewster's work on the microscope, 1837, p. 111); and before 1860 our own Charles A. Spencer used fluorspar in one of the combinations of his objectives (Proc. Acad. Nat. Sci., Phila., Vol. LVI (1904), p. 475; Trans. Amer. Micr, Soc., 1901, p. 23).

powers the defect is left or purposely introduced into all the apochromats. It will be seen from the above statement that for projection or for photography the apochromats cannot be used satisfactorily without the ocular to complete the corrections. (See figs. 114–115.)

The over-correction of the ocular necessary to give the greater magnification to the red constituent of the image leads to the position of the red on the outside of the projected (virtual) beam; hence

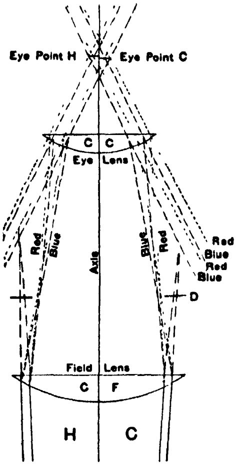

FIG. 115. HUYGENIAN OCULAR SHOWING THE ORDINARY AND THE COMPENSATING ACTION.

(From Spitta, p. 106).

Ordinary action. (*H*).

If the rays are traced on the left, it will be seen that the field lens (*C*) brings the rays to a focus at the diaphragm (*D*), and that they cross and pass on to the eyelens slightly divergent; but in passing through the eyelens (*C*), the red and blue constituents are made parallel to each other, and are projected into the field of vision in close parallel (virtual) bundles and hence appear achromatic.

Compensating action (*C*).

For this the field lens is of flint glass (*F*), and the eyelens of crown glass (*C*). Or the eyelens may be an over-corrected combination. The result is the same, viz., the red image is magnified more than the blue image by the ocular, and this balances the excess magnification of the blue image by the objective, and in the projected (virtual) image the red is on the outside, producing the orange haze at the margin of the field when looking through the ocular, toward a window, or the sky.

in looking through a compensation ocular toward the window or the sky, an orange haze appears around the margin. As the ordinary Huygenian ocular has an under-corrected eyelens, the blue constituent will be on the outside of the projected (virtual) image and there appears a blue haze around the edge of the field (Spitta, pp. 112–113).

ANGULAR AND NUMERICAL APERTURE

§ **261. Angular aperture.** — By this is meant the angle of light which passes from the object to the objective and becomes effective in producing the microscopic image (fig. 116). It has been known for a very long time that the clearness of the image, other things being equal, depends upon the width of the angle of light coming from the object; and that the resolution of details depends very largely upon the angular aperture of the objective. The difficulty of overcoming the aberrations also becomes greater as the angle is increased; and it was the triumph of the early American opticians, Spencer and Tolles, that they were able to make the corrections for high powers with very large angular aperture.

§ **262. Numerical aperture.** — With the introduction of immersion systems into modern microscopy, it was seen and pointed out with great distinctness by Spencer and Tolles that the aperture of such immersion objectives might exceed 180° of light in air. For the average microscopist, however, this seemed an impossibility. By referring to figs. 100–102 the matter becomes very easily intelligible, for it is seen that light in water in passing into air spreads out so that an angle in water of 48° 45′ on each side of the normal (97° 30′) spreads out into an angle of 180° in air; therefore light at an angle of 97° 30′ in water is equal to 180° in air, and if the water immersion objective receives and transmits for the formation of the image an angle of light in the water greater than 97° 30′, its angle is greater than an air angle of 180°. The critical angle for glass to air is 41° on each side of the normal, and a total angle of 82° in the glass would spread out to form the whole 180° in the air. Therefore, if with homogeneous immersion objectives an angle above 82° is transmitted by the objective for the formation of the image, the angle is so much greater than 180° in air.

The confusion was reduced to order by Abbe, to whom makers and users of optical instruments owe so many debts. He applied the simple laws of trigonometry, using the sine function of the angle, and taking into consideration the medium of the lowest refractive index between the object and the objective. If it were air, unity was taken; if water, the index of water — 1.33; if glass, 1.52; and if any other immersion fluid, the refractive index of that fluid. By thus considering the index of refraction of the medium immediately in front of the objective, it becomes possible to make comparisons which are rigidly exact, and express in terms which do not seem to be impossibilities, like an angle in excess of 180° entering a flat surface.

The nomenclature introduced by him and now universally employed is *Numerical Aperture*, and includes in its significance both the angle of the light and the index of refraction of the medium from which the light passes into the objective. The formula is N.A. = $n \sin u$, in which n is the index of refraction of the air for dry, the water for water immersion and the cedar oil for homogeneous immersion; and u, is the sine of half the angle of the light entering the microscope objective, no matter what medium is between the object and objective.

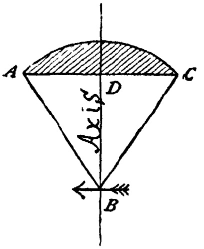

FIG. 116. ANGULAR APERTURE OF AN OBJECTIVE.

Axis, The principal optic axis of the objective.

B The object just outside the principal focus.

A D C Diameter of the front of the objective and base of the angle of aperture.

A B D Half the angle of aperture (u); *AD* representing the sine of u (see § 262).

As there are three factors in this formula, if one knows any two of them the third is readily found.

§ 264. Significance of numerical aperture. — It is now universally agreed that, the corrections in chromatic and spherical aberration being the same, the power to define minute details depends directly on the numerical aperture; the greater the numerical aperture, the greater is the resolution (see also §§ 271–272).

§ 264a. *Table of the usual group of American objectives with their numerical aperture (N.A.) and the method of obtaining it. Compiled from the manufacturers' catalogues.*

Achromatic objectives with initial magnification at 160 mm.	Angular aperture ($2u$)	Natural sine of half the angular aperture (u)	Index of refraction of the medium in front of the objective	Numerical aperture (N.A. = n sin u)
32 mm. (x4)	11°30′	sin 5°45′ = 0.10019 sin u	n = 1.00	N.A. = n sin u = 0.10 +
16 mm. (x10)	29°	sin 14°30′ = 0.25038 sin u	n = 1.00	N.A. = n sin u = 0.25 +
8 mm. (x21 or x20)	60°	sin 30° = 0.5000 sin u	n = 1.00	N.A. = n sin u = 0.50
4 mm. (x43 or 44)	83°	sin 41°30′ = 0.66262 sin u	n = 1.00	N.A. = n sin u = 0.66 +
3 mm. (x60)	116°30′	sin 58°15′ = 0.85035 sin u	n = 1.00	N.A. = n sin u = 0.85 +
1.9 mm. 1.8 mm. oil immersion (x97 or x95)	110°30′	sin 55°15′ = 0.82165 sin u	n = 1.52	N.A. = n sin u = 1.25 −

Table of a dry, a water immersion and a homogeneous immersion objective to give a comparison of the angular aperture required in each to give a uniform N.A. of 0.50.

Dry Objective	60°	sin 30° = 0.5000 sin u	n = 1.00	N.A. = n sin u = 0.50
Water immer. Objective	44°20′	sin 22°10′ = 0.37594 sin u	n = 1.33	N.A. = n sin u = 0.50
Homo. immer. Objective	38°28′	sin 19°14′ = 0.328947 sin u	n = 1.52	N.A = n sin u = 0.50

§ 264b. The values for the index of refraction: n, 1.00 for air; n, 1.33 for water; and n, 1.52 for homogeneous immersion liquid used in determining numerical aperture, are not strictly accurate nor are the sines and numerical apertures; they are approximate round numbers. It will be seen also that in each case the sine of half the angle of aperture may be found by dividing the N.A. by the index of refraction (n) of the medium in front of the objective, for air by 1.00, water by 1.33, and homogeneous liquid, 1.52. It follows also that with dry objectives the N.A. will always be the sine of half the angle of aperture.

§ 265. Why a homogeneous immersion condenser is required. — If the definition of minute details requires adequate numerical aperture, it is evident that it is of fundamental importance that the substage condenser be able to supply the light at the adequate aperture.

Assuming that the substage condenser is properly constructed, the question is, can it illuminate the object with the proper numerical aperture?

By referring to § 262, and to figures 100–102, it is evident that an object mounted on a glass slide and separated from the condenser by a stratum of air can get light from the condenser only up to the critical angle, that is 41°, on each side of the normal, or a total of 82°, corresponding to a numerical aperture of 1. The objective maybe capable, however, of receiving and utilizing a numerical aperture of 1.40.

If now the condenser also has a numerical aperture of 1.40 and it is connected to the slide by means of homogeneous immersion liquid, the entire aperture will illuminate the object and can enter the homogeneous immersion objective.

If the substage condenser is in immersion contact with the glass slip by means of water, then, as shown in figs 100–102, 73, the object can be illuminated with a light cone of 122°, that is, an aperture of $n \sin u^\circ$, in this case $1.52 \times 0.875 = 1.33$ N.A. If the greatest possible aperture is required, as in dark-field illumination (§ 190) and for some of the most exacting work with the bright-field microscope, then the condenser must be in homogeneous immersion contact with the glass slip (figs. 73, 84).

§ **266. Determination of the aperture of objectives with an apertometer.** — Excellent directions for using the Abbe Apertometer may be found in the Jour. Roy. Micr. Soc., 1878, p. 19, and 1880, p. 20; in Dippel, Czapski and Spitta, Chapter XIV. The following directions are but slightly modified from Carpenter-Dallinger, pp. 394–396. The Abbe apertometer involves the same principle as that of Tolles, but it is carried out in a simpler manner; it is shown in fig. 117. As seen by this figure it consists of a semicircular plate of glass. Along the straight edge or chord the glass is beveled at 45°, and near this straight edge is a small, perforated circle, the perforation being in the center of the circle. To use the apertometer the microscope is placed in a vertical position, and the perforated circle is put under the microscope and accurately focused. The circular edge of the apertometer is turned toward a window or plenty of artificial light so that the whole edge is lighted. When the objective is focused on the perforated circle, the draw-tube is removed and in its lower end is inserted the special objective which accompanies the apertometer. This objective and the ocular form a low power compound microscope, and with it the back lens of the objective, whose aperture is to be measured, is observed. The draw-tube is inserted and lowered until the back lens of the objective is in focus, — "In the image of the back lens will be seen stretched across, as it were, the image of the circular part of the apertometer. It will appear as a bright band, because the light which enters normally at the surface is reflected by the bevel part of the chord in a vertical direction so that in reality a fan of 180° in air is formed. There are two sliding screens seen on either side of the apertometer; they slide on the vertical circular portion of the instrument. The images of these screens can be seen in the image of the bright band. *These screens should now be moved so that their edges just touch the periphery of the back lens.* They act, as it were, as a diaphragm to cut the fan and reduce it, so that its angle just equals the aperture of the objective and no more."

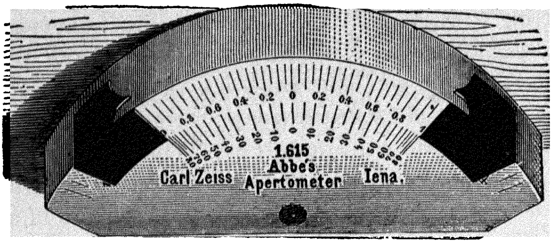

FIG. 117. ABBE APERTOMETER.

As shown in the figure the face bears two series of figures. Those at the top give the numerical aperture, and the lower ones give the angular aperture. It will be noted that there is no angular aperture greater than that represented by a numerical aperture of 1, the sine of 90°.

Determination of numerical aperture (N.A.) by means of a thick plate glass. (H. P. Gage.)

For this the apparatus needed is: (1) A microscope with the objectives and the condenser to be tested.

(2) A strong illuminating device like one of the dark-field lamps (figs. 79–82, 46), or direct sunlight may be used if it is available.

(3) A slip of plate glass 5 to 10 mm. thick and face of 37 × 75 mm. One face of the glass should be given a matt surface with the finest carborundum or emery (§ 95a). The other face should be left smooth.

(4) A pinhole, opaque disc about 10 mm. in diameter should be cemented to the middle of the smooth surface with Canada balsam. This then should be covered, and in balsam, something like a tissue section (§ 533). Tin foil or dense black paper may be used for the opaque disc, and a sewing needle or small pin can be used to make the central opening.

(5) Fine dividers and a scale such as is used in determining the magnification of the microscope (§ 364) for measuring the diameter of the light cone.

The thickness of the glass slip should be known. It is most easily and accurately determined by one of the micrometer calipers (figs. 219–220). The refractive index of the glass slip must also be known. If a refractometer is at hand, it takes but a few minutes to find out the refractive index. If a refractometer is not available an index of 1.515 may be assumed as a sufficiently close approximation.

§ **267. Obtaining the data for determining the N.A. of objectives.** — Place the slip of plate glass on the stage of the microscope with the pinhole disc up. Focus the pinhole with the objective to be tested, then clamp the slip so that it will not move. Make the microscope horizontal. Remove the ocular and point the tube directly toward the lamp. Remove the condenser and the mirror. Mutually arrange the lamp and the microscope till there is seen a circle of light on the ground surface of the slip. With the fine adjustment, focus sharply the pinhole. One can tell when the sharpest focus is gained by the diameter of the circle of light for then it will be greatest. Measure the diameter with the dividers. If one works at night or in a darkened room greater exactness will be possible.

If a homogeneous immersion objective is to be tested, homogeneous liquid must be used to make immersion contact with the cover-glass.

The data thus obtained give all that is needed for finding the numerical aperture, $n \sin u = \text{N.A.}$, for the diameter of the light cone gives the diameter, *A–C*., of the cone and hence the base of the angle (fig. 116). The thickness of the plate glass gives the height (fig. 116, *A–C*, *B–D*). The refractive index by observation is 1.515.

§ **268. Aperture of a condenser.** — For this the plate glass slip is turned over bringing the pinhole down. It is put in immersion contact with the top of the condenser. The microscope is made vertical, and with the plane mirror a strong light is reflected to the condenser. The pinhole should be at the focus of the condenser. To do this it may be necessary in order to get sufficient distance between the top of the condenser and the pinhole, to place a glass slip under the plate glass. The slip must be in immersion contact both with the plate glass and with the condenser. It is well to try first a slide not over 1 mm. thick. In order to make sure that the pinhole is at the focus of the condenser, the body tube of the microscope is removed and light is thrown straight down through the plate glass and pinhole to the condenser. By turning the plane mirror at the proper angle the image of the pinhole will be seen and one can tell whether or not the pinhole is in focus by the sharpness of the edges. If it is not in focus because too low, then a glass slip must be added. If one has already added a glass slip the pinhole may be too high.

Measuring the diameter of the light cone. It is not easy to see the light cone by looking directly down for the light in line of the pinhole is so brilliant. By looking obliquely the border of the cone can be seen.

§ **268a. Determination of the N.A. after the above data have been secured.** — Referring to fig. 116, let *BA* and *BC* be the limiting rays of the light cone from the objective or condenser after passing the pinhole. The thickness of the plate glass, *BD*, and the diameter of the bright disc A.C. have been measured.

The angular aperture of the objective or condenser in glass is *ABC*, and the half angle is *ABD*. $\frac{AD}{DB}$ is the tangent of this half angle, i.e., $\frac{a'}{2}$, or if the thickness of the glass is t and the diameter of the light spot (*AC*) d, then tangent $\frac{a'}{2}$ equals $\frac{d}{2t}$. From trigonometric tables the value of sin $\frac{a'}{2}$ is found corresponding to tangent $\frac{a'}{2}$. This is multiplied by the index of refraction, and the result will be the numerical aperture.

Examples. Suppose the thickness of the plate glass slip is 10 mm.; the diameter of the light disc 5 mm., then tan $\frac{a}{2}$ is $\frac{5}{10 \times 2}$ or .25. The angle whose tangent is .25 is 14°2′ and the sine of this angle is .2425. If this is multiplied by the refractive index of the plate glass: .2425 × 1.515, equals .3673, the numerical aperture of the dry objective in glass. To find the angular aperture in air, find the angle corresponding to the sine .3673. It is the sine of 21° 33′, that is, half the air angle. If this is multiplied by 2 there will result the total air angle (21° 33′ × 2 = 43° 6′).

In an actual experiment it was found that the thickness of the plate glass was 6.7 mm.; its refractive index 1.5135. The diameter of the disc of light obtained from a condenser in immersion contact with the plate glass, is 22.4 mm. Then as above: $\frac{22.4}{2 \times 6.7}$ equals 1.672, which is the tangent of 59° 7′. The sine of 59° 7′ = .8582. This multiplied by the refractive index of the plate glass gives: .8582 × 1.5135 equals 1.30, the N.A. of the condenser. It will be noted that the ray proceeding at an angle of 59° 7′ from the axis in glass would be totally reflected at the glass surface and would never have got into the plate glass in the first place if it had not been in immersion contact with the condenser. Also the sine of 59° 7′, .8582 multiplied by the refractive index of the glass gives a number, 1.30, that is, greater than unity, greater than is possible for any angle, and no ray corresponding to this can exist in air.

§ **269. Refractometer tests upon various liquids.** — In order to investigate adequately the optical properties of the liquids used for homogeneous immersion and other purposes in microscopy, it is necessary to employ a refractometer, and to test all at the same temperature. The one used by the writer for the data given in the following table was loaned to him by Dr. Chamot of the chemical department.

The refractive indices are all at 20° centigrade and for the *D* or sodium line of the solar spectrum (fig. 123); and the average separation into colors between the lines *F* in the green-blue, and *C* in the red of the solar spectrum.

For homogeneous immersion liquid nothing has been found up to the present as satisfactory as thickened cedar-wood oil from *Juniperus virginiana*. Practically all modern homogeneous immersion objectives are designed for use with this immersion liquid, which has an average refractive index at the *D* line of: $n_D = 1.51565$. The average dispersion of these 8 samples is: $vF - vC = 0.01080$.

The first substance purposely employed for homogeneous immersion with objectives by Tolles was Canada balsam from the balsam-fir (*Abies balsamea*). Its index of refraction is somewhat greater and its dispersion somewhat smaller than that of thickened cedar-wood oil, but in case cedar oil is not available it might still be used with successful results.

It will be seen by consulting the table that there is no single liquid which can take the place of cedar-wood oil for immersion purposes. Different workers have found the viscidity of the cedar oil a disadvantage for, in examining preparations in thin liquids, the cover-glass is likely to be pulled about by the adhesion of the cedar oil to the objective. In looking for a substitute in which the viscosity would be less, the heavy mineral oils of the paraffin and naphthalene series have come into use (§ 309). Their refractive index and dispersion are somewhat different from cedar oil so that they do not make a perfect substitute alone, but mixed with alpha-bromonaphthalene they can be brought to the same refractive index, but not exactly to the same dispersion. Such a mixture answers for most purposes and they have the advantage of not being volatile and of having little viscidity. Their odor, however, is not so pleasant as cedar oil. In passing, it might be said that the homogeneous immersion objectives may be used without any immersion liquid, or with water, with castor oil, glycerin, etc. It should never be forgotten, however, that for the best effects one must employ an immersion liquid for which the optician corrected the objective.

Table Showing the Index of Refraction at the D line (^nD) *and the Mean Dispersion* ($^nF-{}^nC$) *between the Fixed Lines F and C, and the v-value* $\frac{^nD-1}{^nF-{}^nC}$ *With Various Homogeneous Liquids and Other Substances.*

Name of Substance	Index of Refraction (^nD)	Mean Dispersion ($^nF-{}^nC$)	*v*-Value of Dispersion $\frac{^nD-1}{^nF-{}^nC}$
(A) Homogeneous Cedar Oil	1.5190	0.01125	46.13
(B) " " "	1.5161	0.01072	48.14
(C) " " "	1.5166	0.01065	48.50
(D) " " "	1.5130	0.01089	47.10
(E) " " "	1.5132	0.01066	48.13
(F) " " "	1.5129	0.01082	47.37 +
(G) " " "	1.5145	0.01085	47.42 −
(Ga) " " "	1.5178 cr. 1.5178 cl.	0.01070 0.01066	48.40 48.52
Averages for Cedar Oil	1.51565	0.01080	47.74
(H) BrN 18% in mineral oil, Naph.	1.5152	0.01082	47.60
(J) BrN 17.25% in mineral oil, P.	1.5151	0.01270	40.55
Aqua distillata (H_2O)	1.33338	0.00582	57.44
(Tap water) (H_2O)	1.33365	0.00626	53.30
Alpha-Bromo-Naphthalene ($C_{10}H_7Br$.)	1.5586	0.013485	41.40
Canada balsam, Pennock's paper-filtered	1.5202	0.00958	54.30
Canada balsam thinned with xylene	1.51578	0.00928	55.58
Carbon tetrachlorid (CCl_4)	1.4614	0.00983	46.89
Castor oil (Oleum ricini)	1.4795	0.00892	53.79
Cedarwood oil (Florida extra)	1.5035	0.01064	47.32
Cedarwood oil, very thick	1.5203	0.01072	48.53
Chloroform ($CHCl_3$)	1.4462	0.00892	50.02
Clove oil (Oleum caryophylli) old	1.5399	0.01725	31.30
Glycerin ($C_3H_5(OH)_3$)	1.4720	0.00816	57.73
Nujol (mineral oil)	1.4789	0.00885	54.11
Petrolatum liquidum, Parf.	1.48525	0.00875	55.47
Petrolatum liquidum, Naph.	1.4840	0.00898	53.89
Sandal wood oil	1.52	0.011	
Turpentine (commercial)	1.4749	0.01071	44.30
Xylene (C_8H_{10}) pure	1.4965	0.01532	32.41
Xylene (commercial)	1.4934	0.01533	32.12

If the immersion cedar oil gets too thick, the best substance for thinning it is the thin cedar-wood oil (Florida extra).

§ **270. Diffracted light in microscopy.** — As most microscopic observation depends upon directed light from some source like the sun or a lamp sent to and through the object by a mirror only or by the aid of a condenser or a mirror and condenser, the phenomena of diffraction are present. It is evident that if the objects observed were self-luminous the conditions would be different from those existing when the object must be viewed with direct light from some outside source.

In traversing small orifices or slits and objects with minute details the spreading out of diffracted light is a necessary accompaniment. The diffracted rays are shown by broken lines in the accompanying figures from Wright (fig. 118). As seen from these, there may be two systems of diffracted rays, one from the object and another from the border of the objective, and these two systems of diffracted rays act differently.

The rôle played by the diffracted light has been variously interpreted by opticians. By Abbe and his adherents diffracted light is of supreme importance, and microscopic vision is a thing by itself (*sui generis*) and not to be interpreted by ordinary geometric optics. Certain very striking experiments have been devised to show the accuracy of this hypothesis, but, as pointed out by many, the ordinary use of the microscope never involves the conditions realized in those experiments.

While the supreme importance ascribed by some to the diffracted light may not be accepted, no one will deny the presence of diffraction phenomena in microscopic vision. If, furthermore, the diffracted rays are brought by the microscope to the final focus with the undiffracted light passing from the object through the microscope, the image will be conceivably more perfect than as if the diffracted rays produce secondary images, or mere blur.

§ **271. Depths of focus and aperture.** — It is known to all workers with the microscope that with objectives of low aperture it is possible to change the focus rather markedly up or down without seeming to lose in sharpness, while with objectives of great aperture a sharp focus is almost immediately lost in focusing up or down beyond a point. The reason for this is made strikingly evident by

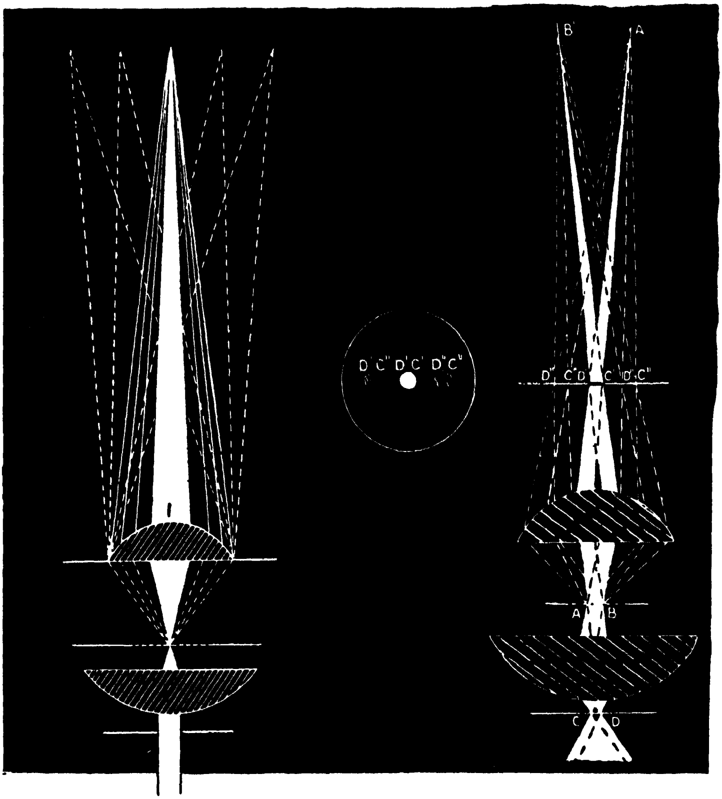

FIG. 118. DIFFRACTED LIGHT IN MICROSCOPY.

(From Wright's Principles).

Object (grating) lighted with a narrow beam (*I*) from the condenser and giving off diffracted rays which are brought to a focus with the dioptric beam (I) above the objective in part (full lines); and in part forming diffracted beams on each side above the objective (broken lines). These diffracted beams not brought to the same focus as the dioptric beam cause imperfections or confusion in the image. Small diaphragm (*C D*) below the condenser focused on the grating, *A B*, and from this point the dioptric beam (solid white) and diffracted light (broken lines) extend through the objective and finally focus at *B′ A′*. By looking at the eye-point with a magnifier the image of the back lens shows not only the diaphragm image (*D′ C′*), but secondary images of the same (*D′ C″* and *D″ C″*). See small figure in the middle also.

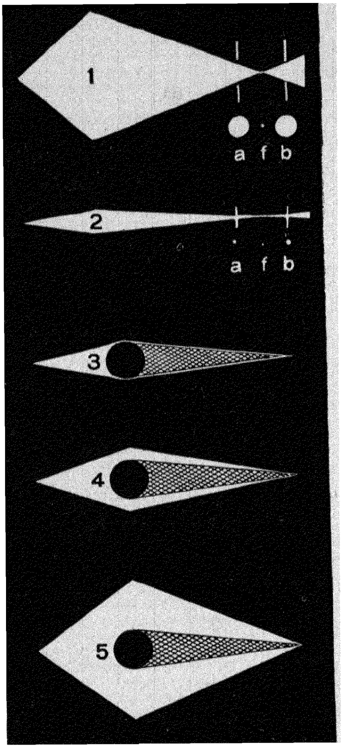

FIG. 119. EFFECT OF APERTURE ON DEFINITE FOCUS AND ON OVERCOMING OPACITIES.

(From Wright's Principles of Microscopy, p. 77).

(1) To show the definiteness of the focus (*f*) with a large aperture. Either above or below this is a large diffusion circle (*a b*) due to the size of the section of the aperture.

(2) Indefiniteness of the focus due to the fact that a cross section of the aperture considerably above or below the true focus (*f*), gives so small a diffusion circle (*a* or *b*), that it can hardly be distinguished from the true focus.

(3) Low aperture and an opacity in the path of the light. It is so large relatively here that a clear image would be impossible.

(4) The same opacity in a larger aperture.

(5) The same opacity in a still larger aperture. There is now enough of the beam outside the opacity to make the object visible.

fig. 119 1, 2. Let *f* be the most perfect focus; if one turns to *a* or *b* the appearance is almost unchanged in the low apertured objective (2), but the diffusion circle is very marked in the high apertured objective (1). Furthermore, the brilliancy of the image must be markedly greater with the larger aperture (Wright, p. 77).

§ **272. Aperture and the effect of opacities.** — Between the retina and the object there are many possibilities of opacities in the image-producing beam of light — for example, the eye lashes, particles of dirt in the tears over the cornea, besides particles on the glass surfaces. Figure 119 3, 4, 5 show graphically the relative obscuration which must result with the same opacity in beams of different aperture. In (3) the shadow is so great that almost the entire aperture is obscured, and vision made difficult or impossible. In (4) with a larger aperture the shadow is not so overwhelming, and in (5) with the large aperture there is still possibility of fairly good vision in spite of the shadow.

It is believed that the inevitable narrowing of the beam in high power magnification and the presence of opacities in the eye form the bar to resolution, and that if the apparatus and the eye could, on the one hand, be free from opacities to throw shadows and thus obscure the image, or on the other hand the terminal beam could be opened up to make the aperture greater, the eye could discriminate beyond the limits heretofore ascribed to it (Wright, Ch. XVI).

As the higher the power of the ocular the smaller is the eyepoint (figs. 24–25), it is evident that any obscurities have a greater effect with the high ocular. The rule to use as low an ocular as possible is a good one to follow with bright field illumination (Wright, p. 227).

For dark-field illumination, the high power oculars are mostly better (§ 181).

Consult Carpenter-Dallinger and Beck, Part II, Chamot and Spitta, and Sir. A. E. Wright for further information.

CHAPTER V

MICRO-SPECTROSCOPE; POCKET SPECTROSCOPE
§§ 273-302; FIGURES 120-124

RADIATION FROM THE SUN AND OTHER SOURCES

§ **273. Visible and invisible radiation.** — From any primary source of light-energy like the sun, the electric arc, etc., not only is given off the energy which to the eye is appreciated as light, but wave lengths of energy both longer and shorter than those affecting the eye. As shown in fig. 93, the segment of the energy spectrum which is visible to the eye is exceedingly limited, being included between about $\lambda 0.4\mu$ and $\lambda 0.7\mu$. Under special illumination, waves shorter than $\lambda 0.4\mu$ and longer than $\lambda 0.7\mu$ can be seen, but the extension into the infra-red or the ultra-violet is slight, and is not used for ordinary visual purposes.

It is fortunate for optical instruments that the visible spectrum is so limited. Indeed, if the visible spectrum were even more limited, as shown by the use of monochromatic light, it would be easier to obtain perfect images, for the aberrations arising from the different wave lengths would be avoided.

The spectroscope has for its object the giving of information concerning the visible spectrum, and it has proved of great help indeed. It should not be forgotten, however, that the color effects produced by the spectroscope are not the only ones and in some ways not the most important. What it really does is to divide the wave lengths into groups, and in absorption phenomena the important thing is that some wave lengths are not present or are cut out by the absorbing medium and hence there are present dark bands in the spectrum (absorption bands). These absorption bands could be seen and their significance appreciated by a person wholly color blind — and there is occasionally such a person.

§ **274. A micro-spectroscope,** spectroscopic or spectral ocular, is a direct-vision spectroscope combined with a microscope ocular of the Huygenian form. At the usual position of the ocular diaphragm is substituted a special slit mechanism. The spectroscope part of the combination consists of an Amici prism of considerable dispersion placed in a tube hinged to the top of the ocular and fastened by a spring. This makes it possible to swing the spectroscope aside and look into the ocular in the usual way. In making spectroscopic observations, the spectroscope is brought over the ocular in the line of sight.

The spectroscope is made complete by the eyelens of the ocular and the slit mechanism in place of the ocular diaphragm. This slit should be parallel to the apices of the prisms which are located at the focal point of the eyelens. Light traversing the slit is rendered approximately parallel by the eyelens of the ocular.

At the diaphragm level is a prism for reflecting horizontal rays vertically. This device is for a comparison spectrum side by side with the spectrum of the object on the stage of the microscope.

Finally, near the top is a lateral tube with mirror for the purpose of projecting a scale of wave lengths upon the spectrum under observation.

In this Amici prism the excess dispersion is given by the flint glass prism or prisms, and the parallelization by the crown glass prisms; and following the rule that the shortest waves are bent most, the colors have the position indicated in figure 124. But if one looks into the direct vision spectroscope or holds the eye close to the single prism (fig. 120), the colors will appear reversed as if the red were more bent. The explanation of this is shown in fig. 120, 2, where it can be readily seen that if the eye is placed at *E*, close to the prism, the different colored rays appear in the direction from which they reach the eye and consequently are crossed in being projected into the field of vision and the real position is inverted. The same is true in looking into the micro-spectroscope. The actual position of the different colors may be determined by placing some ground-glass or some of the lens-paper near the prism and observing with the eye at the distance of distinct vision.

§ 274a. The author wishes to acknowledge the aid rendered by Professor E. L. Nichols in giving the explanation offered in § 274.

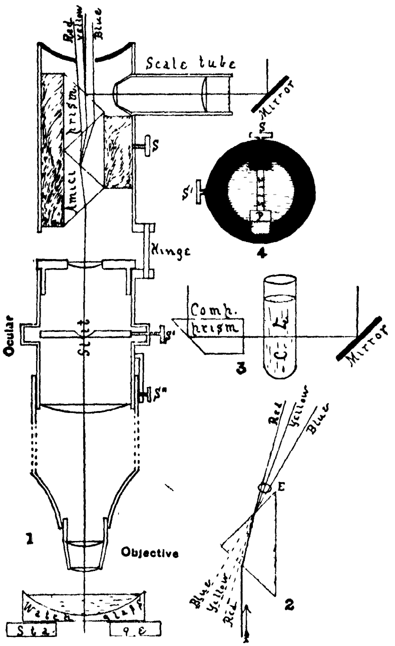

FIG. 120. DIAGRAM OF A DIRECT-VISION MICRO-SPECTROSCOPE.

1 The spectroscope is shown in position on the microscope, the tube of the microscope being much shortened to save space.

Stage, the stage of the microscope on which is a watch glass with sloping sides.

Objective The objective of the microscope.

S S' S" Screws for clamping the apparatus and for changing the position of parts.

Slit The slit of the spectroscope between the ocular lenses in the position of the ocular diaphragm, i.e. where the real image of the object to be examined is formed.

Hinge The hinge on which the prism can be turned off the ocular.

Amici prism The direct-vision prism composed of a middle flint and two crown-glass prisms.

Red Yellow Blue Arrangement of the colors as they emerge from the prism.

Scale tube and Mirror The mirror to throw light into the scale tube and project an image of the Angström scale into the field.

2 *Prism* showing that with the eye close to the prism the colors seem reversed from the position actually occupied.

3 *Comp. prism* The prism introduced under the slit and serving to reflect up into the microscope a spectrum for comparison with that extending along the axis of the microscope from below. *C L* Liquid in the tube whose spectrum is to be compared with that of the liquid in the watch glass on the stage of the microscope.

4 The slit mechanism and comparison prism (*p*).

S S Set screws for changing the width and length of the slit.

VARIOUS KINDS OF SPECTRA

By a spectrum is meant the colored bands appearing when the light traverses a dispersing prism or comes from a diffraction grating, or is affected in any way to separate the different wave lengths of light into groups. When daylight or some good artificial light is thus dispersed one gets the appearance so familiar in the rainbow.

§ **275. Continuous spectrum.** — In case a good artificial light, as the electric light, is used, the various rainbow or spectral colors merge gradually into one another in passing from end to end of the spectrum. There are no breaks or gaps.

§ **276. Line spectrum.** — If a gas is made incandescent, the spectrum it produces consists, not of the various rainbow colors, but of sharp, narrow, bright lines, the color depending on the substance.

All the rest of the spectrum is dark. These line spectra are very

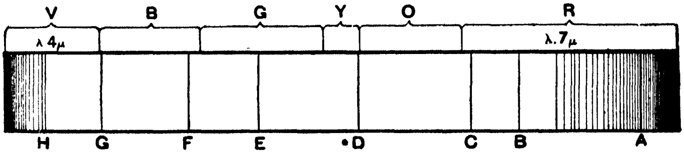

FIG. 121. NORMAL SPECTRUM OF DAYLIGHT SHOWING THE SEGMENTS OF COLOR, *V B G Y O R*, AND THE DARK LINES, *H G F E D C B A*.

In the normal spectrum produced by a grating the refraction is directly proportional to the wave length of the light; here the red is a broad band and the violet-blue narrow. (Compare the prismatic spectrum where the red is narrow and the blue broad.)

$\lambda 0.4\mu$ $\lambda 0.7\mu$, the wave lengths between which the radiation is visible (see fig. 144).

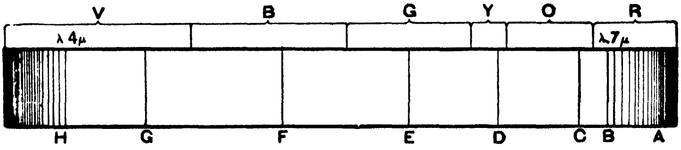

FIG. 122. PRISMATIC SPECTRUM OF DAYLIGHT.

As glass does not refract the different wave lengths in direct proportion to their frequency, the width of the bands of color are strikingly unlike those of a normal spectrum. That is, in a glass spectrum the blue-violet forms a relatively broad band and the red a narrow one.

strikingly shown by metallic vapors heated to incandescence, e.g. sodium. These spectra are usually obtained by heating some salt of the substance (see § 287).

§ **277. Absorption spectrum.** — By this is meant a spectrum in which there are dark lines or bands. The most striking and interesting of the absorption spectra is the Solar Spectrum, or spectrum of sunlight. If this is examined by a good spectroscope it will be found to be crossed by dark lines, the appearance being as if one were to draw pen marks across a continuous spectrum at various levels, sometimes apparently between the colors and sometimes in the midst of a color. These are the so-called Fraunhofer lines. Some of the principal ones have been lettered with Roman capitals, A, B, C, D, E, F, G, H, commencing at the red end. The meaning of these lines was for a long time unknown, but it is now known that they correspond with the bright lines of a line spectrum. For example, if sodium is put in the flame of a spirit or Bunsen lamp it will vaporize and become luminous. If this light is examined there will be seen one or two bright yellow bands corresponding in position with *D* of the solar spectrum (figs. 121, 123). If now the spirit-lamp flame, colored by the incandescent sodium, is placed in the path of the electric light, and it is examined as before, there will be a continuous spectrum, except for dark lines in place of the bright sodium lines. That is, the comparatively cool yellow light of the spirit-lamp cuts off or absorbs the intensely hot yellow light of the electric light; and although the spirit flame sends a yellow light to the spectroscope, it is so faint in comparison with the electric light that the sodium lines appear dark. It is believed that in the sun's atmosphere there are incandescent metal vapors (sodium, iron, etc.), but that they absorb the light from the sun which corresponds with their own wave lengths, and hence the dark lines. If the incandescent vapors could be seen by themselves without the intense light behind them, they would give bright lines as shown by the bright sodium lines seen in the alcohol or Bunsen flame.

§ **278. Absorption spectra from colored substances.** — While the solar spectrum is an absorption spectrum, the term is more commonly applied to the spectra obtained with light which has passed through or has been reflected from colored objects which are not self-luminous.

It is the special purpose of the micro-spectroscope to investigate the spectra of colored objects which are not self-luminous, i.e., blood and other liquids, various minerals, as monazite, etc. The spectra obtained by examining the light reflected from these colored bodies or transmitted through them possess, like the solar spectrum, dark lines or bands, but the bands are usually much wider and less sharply defined. Their number and position depend on the substance or its constitution (fig. 123), and their width, in part, upon the thickness of the body. With some colored bodies, no definite bands are present. The spectrum is simply restricted at one or

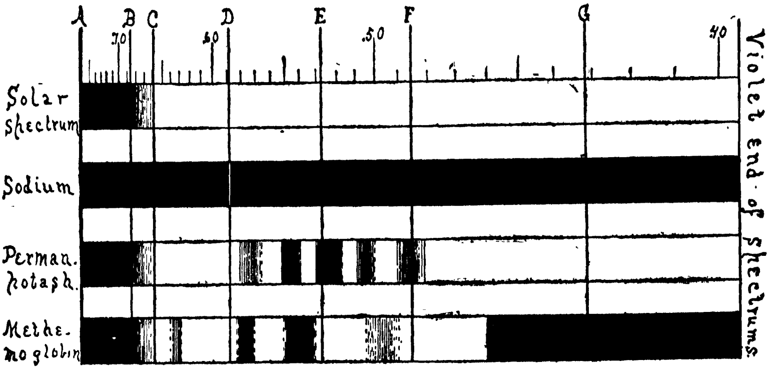

FIG. 123. SPECTRA TO SHOW DIFFERENT KINDS OF ABSORPTION BANDS.

Solar Spectrum The spectrum of daylight showing the dark, fixed lines (Fraunhofer lines) *A B C D E F G*, and the wave lengths in microns, .70, .60 .50, .40.

Sodium The spectrum of incandescent sodium. With this spectroscope it is a single bright yellow band (*D*) at about $\lambda 0.59\mu$, all the rest of the spectrum being dark.

Perman. potash The spectrum of a solution of permanganate of potash. It has five absorption bands, two being especially dark and sharply outlined.

Methaemoglobin The spectrum of methaemoglobin with several absorption bands, the two in the yellow-green being darkest. The blue end of the spectrum is also greatly shortened.

These spectra have the blue end at the right instead of at the left (compare figs. 121–122.

both ends and various of the other colors are considerably lessened in intensity. This is true of many colored fruits.

§ 279. Angström and Stoke's law of absorption spectra. — The waves of light absorbed by a body when light is transmitted through some of its substance are precisely the waves radiated from it when it becomes self-luminous. For example, a piece of glass that is yellow when cool gives out blue light when it is hot enough to be self-luminous. Sodium vapor absorbs two bands of yellow light (*D* lines); but when light is not sent through it, but the vapor itself is luminous and is examined as a source of light, its spectrum gives bright sodium lines, all the rest of the spectrum being dark (fig. 123).

§ 280. Law of color. — The light reaching the eye from a colored solid, liquid, or gaseous body lighted with white light will be that due to white light less the light waves that have been absorbed by the colored body. For example, a thin layer of blood under the microscope will appear yellowish green, but a thick layer will appear pure red. If now these two layers are examined with a micro-spectroscope, the thin layer will show all colors, but the red end will be slightly, and the blue end considerably, restricted, and some of the colors will appear considerably lessened in intensity. Finally, there may appear two shadow-like bands, or, if the layer is thick enough, two well-defined dark bands in the green (§ 295).

If the thick layer is examined in the same way, the spectrum will show only red with a little orange light, all the rest being absorbed. Thus the spectroscope shows which colors remain, in part or wholly, and it is the mixture of this remaining or unabsorbed light that gives color to the object.

§ 281. Complementary spectra. — While it is believed that Angström's law (§ 280) is correct, there are many bodies on which it cannot be tested, as they change in chemical or molecular constitution before reaching a sufficiently high temperature to become luminous. There are compounds, however, like those of didymium, erbium, and terbium, which do not change with the heat necessary to render them luminous, and with them the incandescent and absorption spectra are mutually complementary, the one presenting bright lines where the other presents dark ones (Daniell).

Adjusting the Micro-spectroscope

§ **282. The micro-spectroscope,** or spectroscopic ocular, is put in the place of the ordinary ocular in the microscope, and clamped to the top of the tube by means of a side screw for the purpose.

§ **283. Adjustment of the slit.** — In place of the ordinary diaphragm with circular opening, the spectral ocular has a diaphragm composed of two movable knife edges by which a slit-like opening of greater or less width and length may be obtained at will by the use of screws for the purpose. To adjust the slit, depress the lever holding the prism-tube in position over the ocular, and swing the prism aside. One can then look directly into the ocular. The lateral screw should be used, and the knife edges approached till they appear about half a millimeter apart. If now the Amici prism is put back in place and the microscope well lighted, one will see a spectrum by looking into the upper end of the spectroscope. If the slit is too wide, the colors will overlap in the middle of the spectrum and be pure only at the red and blue ends; and the Fraunhofer or other bands in the spectrum will be faint or invisible. Dust on the edges of the slit gives the appearance of longitudinal streaks on the spectrum.

§ **284. Mutual arrangement of slit and prism.** — In order that the spectrum may appear as if made up of colored bands going directly across the long axis of the spectrum, the slit must be parallel with the refracting edge of the prism. If the slit and prism are not thus mutually arranged, the colored bands will appear oblique, and the whole spectrum may be greatly narrowed. If the colored bands are oblique, grasp the prism tube and slowly rotate it to the right or to the left until the various colored bands extend directly across the spectrum.

§ **285. Focusing the slit.** — In order that the lines or bands in the spectrum shall be sharply defined, the eyelens of the ocular should be accurately focused on the slit. The eyelens is movable, and when the prism is swung aside it is easy to focus the slit as one focused for the ocular micrometer (§ 375). If one now uses daylight there will be seen in the spectrum the dark Fraunhofer lines (figs. 121, 123).

To show the necessity of focusing the slit, move the eyelens down or up as far as possible, and the Fraunhofer lines cannot be seen. While looking into the spectroscope move the ocular lens up or down, and when it is focused, the Fraunhofer lines will reappear. As the different colors of the spectrum have different wave lengths, it is necessary to focus the slit for each color if the sharpest possible pictures are desired.

It will be found that the eyelens of the ocular must be farther from the slit for the sharpest focus of the lines at the red end, than for the sharpest focus of those at the blue end. This is because the wave length of the red is markedly greater than for blue light (figs. 93, 124).

Longitudinal dark lines on the spectrum may be due to irregularity of the slit or to the presence of dust. They are most troublesome with a very narrow slit.

§ **286. Comparison or double spectrum.** — In order to compare the spectra of two different substances one must examine their spectra side by side. This is provided for in the better forms of micro-spectroscopes by a prism just below the slit, so placed that the light entering it from the mirror at the side of the drum shall be totally reflected in a vertical direction, and thus parallel with the rays from the microscope. The two spectra will be side by side, with a narrow dark line separating them. If now the slit is well focused and daylight is sent through the microscope and into the side to the reflecting or comparison prism, the colored bands and the Fraunhofer dark lines will appear directly continuous across the two spectra. The prism for the comparison spectrum is movable and may be thrown entirely out of the field if desired. When it is to be used, it is moved about halfway across the field so that the two spectra shall have about the same width.

§ **287. Scale of wave lengths.** — In the Abbe micro-spectroscope the scale is in a separate tube near the top of the prism and at right angles to the prism-tube. A special mirror serves to light the scale, which is projected upon the spectrum by a lens in the scale-tube. By means of this scale, the wave lengths of any part of the spectrum may be read off directly, after the scale is once set in the proper position, that is, when it is set so that any given wave length on the scale is opposite the part of the spectrum known by previous investigation to have that particular wave length. The point most often selected for setting the scale is opposite the sodium line, where the wave length is, according to Angström, 0.5892μ. In adjusting the scale, one may focus very sharply the dark sodium line of the solar spectrum and set the scale so that the number 0.589 is opposite the sodium or *D* line; or a method that is frequently used and serves to illustrate §§ 276–277, is to saturate some asbestos cloth in a strong solution of common salt (sodium chlorid, NaCl) or bicarbonate of soda ($NaHCO_3$). Heat in a Bunsen or alcohol flame and the incandescent sodium will give the bright *D* lines.

If now ordinary daylight is sent through the comparison prism, the bright lines of the sodium will be seen to be directly continuous with the dark lines at *D* in the solar spectrum (fig. 123). By reflecting light into the scale-tube the image of the scale will appear on the spectrum, and by a screw just under the scale-tube, but within the prism-tube, the proper point on the scale (0.589μ) can be brought opposite the sodium band. All the scale will then give the wave lengths directly. Sometimes the scale is oblique to the spectrum. This may be remedied by turning the prism-tube slightly one way or the other. It may be due to the wrong position of the scale itself. If so, grasp the milled ring at the distal end of the scale-tube and, while looking into the spectroscope, rotate the tube until the lines of the scale are parallel with the Fraunhofer lines. It is necessary in adjusting the scale to be sure that the larger number, 0.70, is at the red end of the spectrum.

The numbers on the scale should be clearly defined. If they do not so appear, the scale-tube must be focused by grasping the outer tube of the scale-tube and moving it toward or from the prism-tube until the scale is distinct. In focusing the scale, grasp the outer scale-tube with one hand and the prism-tube with the other, and push or pull in opposite directions. In this way one will be less likely to injure the spectroscope.

§ **288. Designation of wave length.** — Wave lengths of light are designated by the Greek letter λ followed by the number indicating the length in some fraction of a meter. See fig. 93 where the visible spectrum is indicated as lying between wave lengths $\lambda 0.7\mu$ and 0.4μ. In this book the micron (μ) is taken as the unit as with other minute measurements. Other units are also employed, especially smaller ones so that the wave lengths will appear as whole numbers instead of decimal fractions. (See §§ 380–382).

§ **289. Lighting for the micro-spectroscope.** — Opaque objects are illuminated by placing the microscope in a strong light, or by reflecting light upon them by a mirror, or by the use of a bull's-eye or other condenser. The light from one of the dark-field lamps is excellent. For transparent objects, the amount of the substance and the depth of the color must be considered. As a general rule, it is well to use plenty of light, as that from a substage condenser with a large opening in the diaphragm or with the diaphragm entirely open. For very small objects and thin layers of liquids, it may be better to use less light. One must try both methods in a given case, and learn by experience.

The direct and the comparison spectra should be about equally illuminated. One can manage this by putting the object requiring the greater amount of illumination on the stage of the microscope.

Furthermore, one should be on his guard against confusing the ordinary absorption bands with the Fraunhofer lines when daylight is used. With lamplight the Fraunhofer lines are absent.

§ **290. Objective to use with the micro-spectroscope.** — If the material is of considerable bulk, a low objective (16 mm. 10x) is to be preferred. This depends on the nature of the object under examination, however. In case of individual crystals one should use sufficient magnification to make the real image of the crystal entirely fill the width of the slit. The length of the slit may then be regulated by the screw on the side of the drum, and also by the comparison prism. If the object does not fill the whole slit, the white light entering the spectroscope with the light from the object might obscure the absorption bands.

In using high objectives with the micro-spectroscope one must very carefully regulate the light and sometimes shade the object.

§ **291. Focusing the objective.** — For focusing the objective the prism-tube is swung aside, and then the slit made wide by turning the adjustable screw at the side. If the slit is open one can see objects when the microscope is focused as with an ordinary ocular. After an object is focused, it may be put exactly in position to fill the slit of the spectroscope, then the knife edges are brought together till the slit is of the right width; if the slit is then too long it may be shortened by using one of the mechanism screws on the side, or if that is not sufficient, by bringing the comparison prism farther over the field. If one now replaces the Amici prism and looks into the microscope, the spectrum is likely to have longitudinal shimmering lines. To get rid of these, focus up or down a little so that the microscope will be slightly out of focus.

§ **292. Amount of material necessary for absorption spectra and its proper manipulation.** — The amount of material necessary to give an absorption spectrum varies greatly with different substances, and can be determined only by trial. If a transparent solid is under investigation, it is well to have it in the form of a wedge, then successive thicknesses can be brought under the microscope. If a liquid substance is being examined a watch glass with sloping sides forms an excellent vessel to contain it, then successive thicknesses of the liquid can be brought into the field, as with the wedge-shaped solid. Frequently only a very weak solution is obtainable; in this case it can be placed in a homeopathic vial, or in some glass tubing sealed at the end, then one can look lengthwise through the liquid and get the effect of a more concentrated solution. For minute bodies like crystals or blood corpuscles, one may proceed as described in the previous section. (See also § 302.)

MICRO-SPECTROSCOPE EXPERIMENTS

§ **293.** Put the micro-spectroscope in position, arrange the slit and the Amici prism so that the spectrum will show the various spectral colors going directly across it (§ 284), and focus the slit. This may be done either by swinging the prism-tube aside and proceeding as for the ocular micrometer (§ 375), or by moving the eyelens of the ocular up and down while looking into the micro-spectroscope until the dark lines of the solar spectrum are distinct. If they cannot be made distinct by focusing the slit, then the light is too feeble or the slit is too wide. With the lever move the comparison prism across half the field so that the two spectra shall be of equal width. For lighting, see § 289.

§ **293a. Pocket spectroscope.** — Many of the purposes for which a micro-spectroscope was specially designed, can almost as well be accomplished by a much cheaper, pocket, direct-vision spectroscope (Bleile, Trans. Amer. Micr. Soc. 1900, p. 8). To use this with a microscope, it is clamped in some kind of an adjustable holder like the lens holder, (figs. 17, 127), the ocular of the microscope is removed, and the pocket spectroscope is put over the top of the tube and in line with the optic axis of the microscope. As the slit mechanism and the parallelizing lens form a part of the spectroscope, one has simply to open the slit the right amount and focus it by pulling out the tube of the spectroscope. All the other operations are the same as for the larger micro-spectroscope. The object to be examined is put in the center of the field of the microscope. For this, of course the ocular should be in place. After the ocular is removed, one can adjust the pocket spectroscope so that the object sends the light transmitted through it to the spectroscope. If one has a research lamp like figure 80, the iris of the lamp can be made smaller and larger by closing or opening it. Also the image of this opening focused on the object by the condenser can be made larger or smaller by changing the distance between the lamp and the microscope.

The real image of the object should be at the level of the slit of the spectroscope. This is easily determined by using a piece of ground glass over the upper end of the tube, and focusing until the real image is sharp on the ground glass.

This opening or closing of the iris of the lamp and varying the distance between the lamp and the microscope condenser enables one to enclose the object and exclude outside objects almost as effectively as the arrangements in the micro-spectroscope for shortening and narrowing the slit (§ 291.)

§ **294. Absorption spectrum of permanganate of potash.** — Make a solution of permanganate of potash by putting a few crystals in a watch glass of water. The solution should be of such strength that a stratum of 3 to 4 mm. thickness will be transparent. Place the watch glass under the microscope. Use a 16 mm. (10x) or lower objective and open widely the condenser diaphragm; light strongly. Look into the spectroscope and slowly move the watch glass into the field. Note carefully the appearance with the thin stratum of liquid at the edge and then as it gradually thickens on moving the watch glass still farther along. Count the absorption bands and note particularly the red and blue ends. Compare with the comparison spectrum (fig. 123). For strength of solution see § 292.

§ **295. Absorption spectrum of blood.** — Obtain blood from a recently killed animal, or flame a needle, and after it is cool, prick the finger two or three times in a small area; then wind a handkerchief or a rubber tube around the base of the finger and squeeze the finger with the other hand. Some blood will ooze out of the pricks. Rinse this off into a watch glass partly filled with water. Continue to add the blood until the water is quite red. Place the watch glass of diluted blood under the microscope in place of the permanganate, using the same objective, etc. Note carefully the spectrum. It would be advantageous to determine the wave length opposite the center of the dark bands. This may easily be done by setting the scale properly, as described in § 287. Make another preparation, but use a homeopathic vial instead of a watch glass. Cork the vial and lay it down upon the stage of the microscope. Observe the spectrum. It will be like that in the watch glass. Remove the cork and look through the whole length of the vial. The bands will be much darker, and if the solution is thick enough, only red and a little orange will appear. Reinsert the cork and incline the vial so that the light traverses a very thin layer, then gradually elevate the vial and the effect of a thicker and thicker layer may be seen. Note especially that the two characteristic bands unite and form one wide band as the stratum of liquid thickens. Compare with the following.

Add to the vial of diluted blood a drop or two of ammonium sulphide, such as is used for a reducing agent in chemical laboratories. Shake the bottle gently and then allow it to stand for ten or fifteen minutes. Examine it and the two bands will have been replaced by a single, less clearly defined band in about the same position. The blood will also appear somewhat purple. Remove the cork to admit fresh air, then shake the vial vigorously, and the color will change to the bright red of fresh blood. Examine it again with the spectroscope and the two bands will be visible. After five or ten minutes another examination will show but a single band. Incline the bottle so that a thin stratum may be examined. Note that the stratum of liquid must be considerably thicker to show the single absorption band than was necessary to show the two bands in the first experiment. Furthermore, while the single band may be made quite black on thickening the stratum, it will not separate into two bands with a thinner stratum. In this experiment it is very instructive to have the watch glass of arterial blood under the microscope and the vial of blood to which has been added the ammonium sulphide in position for a comparison spectrum.

The two-banded spectrum is that of oxy-hemoglobin, or arterial blood; the single-banded spectrum of hemoglobin (sometimes called reduced hemoglobin) or venous blood. The respiratory oxygen is present in the two-banded spectrum but absent from the single-banded spectrum. When the bottle was shaken the hemoglobin took up oxygen from the air and became oxy-hemoglobin, as occurs in the lungs, but soon the ammonium sulphide took away the respiratory oxygen, thus reducing the oxy-hemoglobin to hemoglobin. This may be repeated many times (fig. 124).

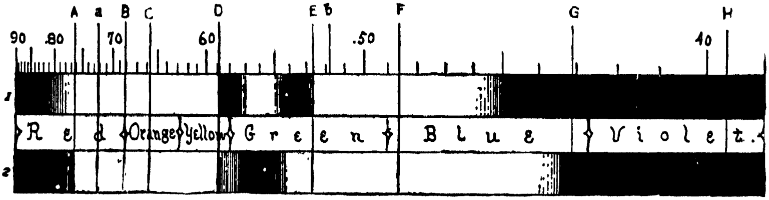

FIG. 124. ABSORPTION SPECTRUM OF ARTERIAL AND OF VENOUS BLOOD. (From Gamgee and McMunn).

1 Absorption of arterial blood, oxy-hemoglobin. There are two definite bands between wave lengths 0.60μ and 0.50μ, that is, in the yellow-green, and the blue end of the spectrum is cut down markedly.

2 Single dark band of the venous blood, hemoglobin, in the yellow-green. The blue end of the spectrum is less cut off than with arterial blood.

A B C D E F G H Fixed lines of the solar spectrum .90, .80, .70, .60, .50, .40; wave lengths in microns in the different regions. These spectra have the red end at the left instead of to the right, as is now more usual (figs. 120–122).

§ **296. Met-hemoglobin.** — The absorption spectrum of met-hemoglobin is characterized by a considerable darkening of the blue end of the spectrum and of four absorption bands, one in the red near the line *C* and two between *D* and *E*, nearly in the place of the two bands of oxy-hemoglobin; finally there is a somewhat faint, wide band near *F*. Such a met-hemoglobin spectrum is best obtained by making the solution of blood in water of such a concentration that the two oxy-hemoglobin bands run together, and then adding three or four drops of a 0.1 % aqueous solution of permanganate of potash. Soon the bright red will change to a brownish color, when it may be examined (fig. 123). Instead of the permanganate one may use hydrogen dioxide (H_2O_2).

§ **297. Carbon monoxide hemoglobin (CO-hemoglobin).** — To obtain this, kill an animal in illuminating gas, or one may allow illuminating gas to bubble through some blood already taken from the body. The gas should bubble through a minute or two. The oxygen will be displaced by carbon monoxide. This forms quite a stable compound with hemoglobin, and is of a bright cherry-red color. Its spectrum is nearly like that of oxy-hemoglobin, but the bands are farther toward the blue. Add several drops of ammonium sulphide and allow the blood to stand some time. No reduction will take place, thus forming a marked contrast to solutions of oxy-hemoglobin. By the addition of a few drops of glacial acetic acid a dark brownish-red color is produced.

§ **298. Carmine solution.** — Make a solution of carmine by putting 0.1 gram of carmine in 100 cc. of water and adding 10 drops of strong ammonia. Put some of this in a watch glass or in a small vial and compare the spectrum with that of oxy-hemoglobin or carbon-monoxide hemoglobin. It has two bands in nearly the same position, thus giving the spectrum a striking similarity to blood. If now several drops, 15 or 20, of glacial acetic acid are added to the carmine, the bands remain and the color is not markedly changed, while with either oxy-hemoglobin or CO-hemoglobin the color is decidedly changed from the bright red to a dull reddish-brown, and the spectrum, if any can be seen, is markedly different. Carmine and O-hemoglobin can be distinguished by the use of ammonium sulphide, the carmine remaining practically unchanged while the blood shows the single band of hemoglobin (§ 295). The acetic acid serves to differentiate the CO-hemoglobin as well as the O-hemoglobin.

§ **299. Colored bodies not giving banded spectra.** — Some quite brilliantly colored objects, like the skin of a red apple, do not give a banded spectrum. Take the skin of a red apple, mount it on a slide, put on a cover-glass, and add a drop of water at the edge of the cover. Put the preparation under the microscope and observe the spectrum. Although no bands will appear, in some cases at least, yet the ends of the spectrum will be restricted and various regions of the spectrum will not be so bright as the comparison spectrum. Here the red color arises from the mixture of the unabsorbed waves, as occurs with other colored objects. In this case, however, not all the light of a given wave length is absorbed; consequently there are no clearly defined dark bands; the light is simply less brilliant in certain regions and the red rays so predominate that they give the prevailing color.

§ **300. Nearly colorless bodies with clearly marked absorption spectra.** — In contradistinction to the brightly colored objects with no distinct absorption bands are those nearly colorless bodies and solutions which give as sharply defined absorption bands as could be desired. The best examples of this are afforded by solutions of the rare earths, didymium, etc. These in solutions that give hardly a trace of color to the eye give absorption bands that almost rival the Fraunhofer lines in sharpness.

§ **301. Absorption spectra of minerals.** — As example, take some monazite sand on a slide and either mount it in balsam, or cover and add a drop of water. The examination may be made also with the dry sand, but it is less satisfactory. Light well with transmitted light and move the preparation slowly about. Absorption bands will appear occasionally. Swing the prism tube off the ocular, open the slit, and focus the sand. Get the image of one or more grains directly in the slit, then narrow and shorten the slit so that no light can reach the spectroscope that has not traversed the grain of sand. The spectrum will be satisfactory under such conditions. It is frequently of great service in determining the character of unknown mineral sands to compare the spectra with known minerals. If the absorption bands are identical, it is strong evidence in favor of the identity of the minerals.

§ **302.** While the study of absorption spectra gives one a great deal of accurate information, great caution must be exercised in drawing conclusions as to the identity or even the close relationship of bodies giving approximately the same absorption spectra. The rule followed by the best workers is to have a known body as control and to treat the unknown body and known body with the same reagents, and to dissolve them in the same medium. If all the reactions are identical, then the presumption is strong that the bodies are identical or very closely related. For example, while one might be in doubt between a solution of oxy- or CO-hemoglobin and carmine, the addition of ammonium sulphide serves to change the double to a single band in the O-hemoglobin, and glacial acetic acid enables one to distinguish between the CO-blood and the carmine, although the ammonium sulphide would not enable one to make the distinction. Furthermore, it is unsafe to compare objects dissolved in different media. Different objects as "cyanine and aniline blue dissolved in alcohol give a very similar spectrum, but in water a totally different one." "Totally different bodies show absorption bands in exactly the same position (solid nitrate of uranium and permanganate of potash in the blue)" (MacMunn). The rule given by MacMunn is a good one: "The recognition of a body becomes more certain if its spectrum consists of several absorption bands, but even the coincidence of these bands with those of another body is not sufficient to enable us to infer chemical identity, what enables us to do so with certainty is the fact, that the two solutions give bands of equal intensities in the same parts of the spectrum which undergo analogous changes on the addition of the same reagent. It should be borne in mind that the position of a band may be changed greatly through increased or diminished dissociation, and that the absorption bands given by a crystal may be quite different from those given by the same material in solution and furthermore that the absorption spectra are sometimes different in different directions through the crystal" (Chamot, p. 112). This is easily demonstrated if one has a centering, revolving stage on the microscope (fig. 92).

For the use of the spectroscope with the ultra-violet microscope for determining the character of fluorescent light, see § 314.

CHAPTER VI

THE ULTRA-VIOLET MICROSCOPE AND PHYSICAL ANALYSIS.

§§ 303-324; FIGURES 125-130

THE ULTRA-VIOLET MICROSCOPE

The necessity of sunlight for green plants and its beneficial effect upon animal life have been the common knowledge of mankind for unnumbered generations.

The knowledge is relatively recent, however, that the radiation from the sun and from artificial sources is composed of waves of varying length, and that only a narrow band of that radiation is visible (fig. 93). The knowledge is still more recent that the short waves beyond the visible spectrum (ultra-violet, x-rays, etc.), have a profound effect upon living things, and that the appearances given to various structures by these invisible waves promise to give much definite information concerning their physical make-up.

So difficult is the complete understanding of the function and structure of organic nature that it seems worth while to make use of every means available to aid in making the structure and the function more completely understood. From what is already known, it is certain that the ultra-violet radiation has not only a profound physiological effect upon animals and plants, but also gives definite appearances to various structures and thus throws light upon their chemical and physical constitution.

The effect that ultra-violet radiation has upon living things, is primarily upon the individual structural units or cells of which they are composed. If then the structural units can be studied before, during and after the ultra-violet treatment by means of a suitable microscope (an ultra-violet microscope) in which all powers can be used, it is hoped that enough of the changes can be discovered to enable the biologist to gain some understanding of the changes that occur in the animal or plant as a whole.

It is already certain knowledge that the ultra-violet microscope is of use in what may be called the *physical analysis* of living things, and of their individual tissues and organs, for, some of them at least, when excited by the short, invisible, ultra-violet radiation, emit visible radiation. In that case the tissues or organs glow with a soft radiance of their own, and become visible by their own luminescence. When they do thus become luminous, they are said to *fluoresce.*

Some structures do not fluoresce when radiated by ultra-violet. Such structures, then, remain dark in ultra-violet. (See §§ 317–318.) Although a structure or organism does not fluoresce, it may be profoundly affected by the ultra-violet radiation. For example, it has been known for decades that bacteria and other microscopic forms are killed by ultra-violet in excess. Physicians also have learned that in applying ultra-violet to animals and to men, the amount must be limited or serious results occur. Every one knows about sun-burn, and how painful it may be. Mountain climbers found long ago that sun-burn was almost sure to occur on the snow- and ice-covered peaks if one did not take the precaution to smear the face with some substance opaque to ultra-violet and to protect the eyes by colored glasses. We think now that it is not so much the visible light that does the damage, but the invisible, ultra-violet.

§ **303. Ultra-violet or fluorescence microscope.** — This is a microscope under which small objects can be submitted to ultra-violet radiation and the results observed.

For such observations there must be, first, some source in which the ultra-violet is abundant. Best for the microscope is the lately perfected high-pressure capillary mercury arc (100-watt H4 lamp) of the General Electric Vapor Lamp Co. (fig. 129 A).

The carbon arc using specially filled cored carbons is also good.

These sources also produce a great amount of visible radiation, which must be excluded by the use of suitable screens so that only the ultra-violet radiation is present in the radiation utilized. It is also highly important to know just what wave lengths pass through the screen so that any observed effect on the object can be ascribed to the wave length, or wave lengths producing the effects. For example, if the object is fluorescent, it is desirable to know what wave-lengths excite the fluorescence.

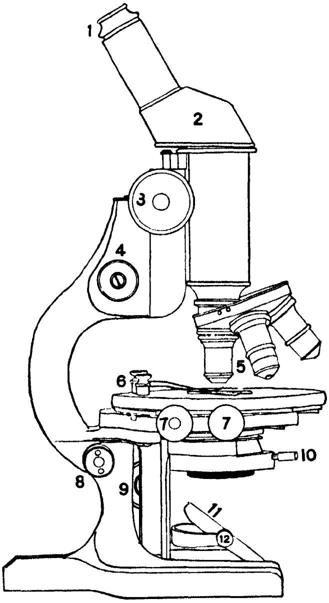

FIG. 125. ULTRA-VIOLET MICROSCOPE.

1 Incline ocular. The usual straight ocular is satisfactory.
2 Housing of the prism to give the inclination.
3 Wheel of the coarse adjustment.
4 Wheel of the fine adjustment.
5 Nosepiece with three objectives.
6 Spring clips for holding the specimen in place.
7–7 Mechanical stage movement screw heads.
8 Joint in the pillar for inclination of the microscope.
9 Jointed pillar.
10 Centering screw head for the condenser.
11 Aluminum vapor mirror for reflecting ultra-violet radiation.
12 Tightening screw head for the mirror mounting.

§ **301. Aluminum vapor mirror.** — Some kind of a reflector is necessary to change the direction of the illuminating beam in microscopic work. The ordinary mirror with silvered back does not answer for ultra-violet reflection for so great a number of the short ultra-violet wave lengths are absorbed by the silver and the glass. Formerly a quartz prism was used as reflector. This is heavy, expensive and rather difficult to manipulate. Since the publication of the 15th edition there has been developed a practical means of making vapor deposits of metals. A first surface mirror on ordinary glass of aluminum has given the author as good results as ever could be obtained by the quartz

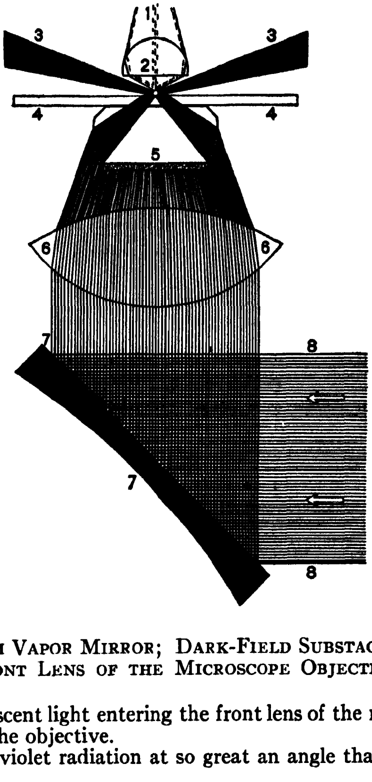

FIG. 126. ALUMINUM VAPOR MIRROR; DARK-FIELD SUBSTAGE CONDENSER, AND FRONT LENS OF THE MICROSCOPE OBJECTIVE.

1 Beam of fluorescent light entering the front lens of the microscope objective.
2 Front lens of the objective.
3 Beam of ultra-violet radiation at so great an angle that it cannot enter the objective.
4 Corex glass slip in immersion contact with the top of the dark-field condenser.
5 The hollow cone of ultra-violet radiation. The central part of the cone is stopped by the opaque diaphragm at the base or lower end of the quartz dark-field element of the condenser.
6 Lower quartz element of the dark-field condenser.
7 The aluminum-vapor mirror on the front of glass or the back of quartz.
8 Beam of ultra-violet radiation.

prism reflector, and is easier to manipulate. To toughen the soft aluminum film there is first deposited a chromium film and on this the aluminum. In spite of the toughening by the chromium, the surface becomes more or less scratched and dimmed by long usage and much cleaning. To have the advantages of a back or second surface mirror on a hard transparent substance, by the efficient aid of Dr. H. P. Gage of the Corning Glass Works and the "The Evaporated Films Company of Ithaca, N. Y." the author has secured aluminum vapor reflectors with the aluminum film on the back of polished quartz discs. These are especially brilliant reflectors of the ultra-violet and the protection of the aluminum film by the quartz covering will, it is believed, preserve the aluminum reflecting surface with its original brilliance indefinitely. From determinations by George B. Sabine of the physics department of Cornell University, the percentage of reflection averaged for the second-surface, quartz mirror, waves 238 to 377 mμ, 89%. Such mirrors are mounted in the usual way for the fork of the mirror holder, or the unmounted mirror can be stuck to the glass mirror with beeswax.

It may be stated in passing, that such aluminum mirrors are excellent for all microscopic work including photography with the microscope.

§ 305. As the ultra-violet is invisible, it is necessary to use some means for knowing when the radiation is directed from the source to the reflector, whence by proper manipulation of the reflector it is sent up through the condenser to the object on the stage of the microscope. A card smeared with a strong emulsion of anthracene in cane sugar and dried, makes a good detector of ultra-violet from its strong fluorescence. See also § 315 to make sure the object is well illuminated.

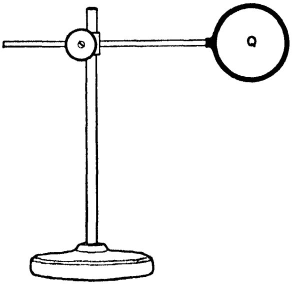

Fig. 127. Bull's-Eye, Quartz Condenser (Q) for Use with the Ultra-Violet Microscope.

§ 306. Ultra-Violet condenser. — The substage condenser and the bull's-eye condenser (fig. 128) must be made of quartz to insure the transmission of the ultra-violet radiation.

§ **307. Quartz dark-field element.** — The upper element of the condenser should be for dark-field (§ 181) illumination. The object will then be radiated with the ultra-violet at so great an angle that none of it will get directly into the microscope objective (§§ 171, 178). If, however, the object is fluorescent, the luminous object will send visible light to the objective and thence on to the eye of the observer. The relatively small amount of ultra-violet which is deflected by the object into the objective will not cause sufficient

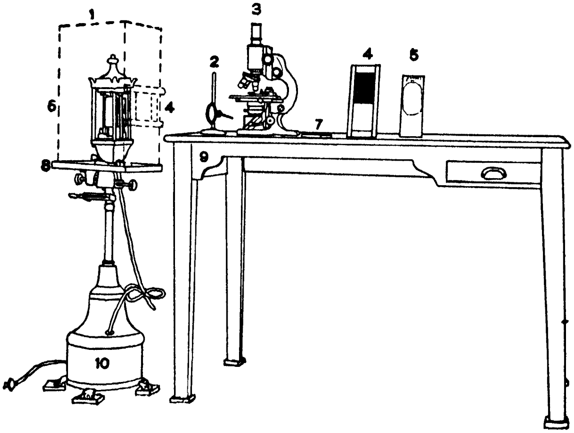

Fig. 128. Arrangement of Parts for the Ultra-Violet Microscope.

1 Outline of the lamp-house

2 The quartz condenser on adjustable stand for directing the ultra-violet radiation from the mercury lamp to the quartz prism of the ultra-violet microscope.

3 Ultra-Violet microscope. Here it is shown with the usual vertical tube for the ocular.

4 Sliding screen carrier. The dark part is the red-purple screen, and the white part the corex, ground glass to transmit the full mercury radiation. It is also shown over the window in the lamp-house.

5 Card with a coating of anthracene to aid in directing the ultra-violet upon the quartz prism.

6 The mercury lamp head opposite the quartz tube of the lamp.

7 Piece of uranium glass on the table. It is one of the most perfect detectors of ultra-violet radiation.

8 Metal shelf for holding the lamp-house.

9 Table for the microscope, etc.

10 Regulating mechanism for the current with the mercury arc. The feet of the lamp and of the table have pads of thick felt under them to diminish vibration.

fluorescence of the glass or of the Canada balsam used in sealing the lenses to interfere. If the regular top element of the condenser is left in place, the amount of ultra-violet getting past the object and into the objective is likely to cause so much fluorescence in the objective that the appearance is like looking into a bright fog.

§ **308. Ultra-Violet transmitting slips.** — For mounting slips one must use a non-fluorescing substance like quartz or corex D glass of the Corning Glass Works (fig. 218). The corex slips, if cut from sheets, cost about $6.00 per hundred while the quartz slips cost from $3.50 to $10.00 each. As the quartz and the corex appear so much like glass, the author has had them made in a special size, 65 × 25 mm. (fig. 218). He found it wise to mark them with a writing diamond also.

For the cover-glasses, those ordinarily used answer well. They transmit the fluorescent light from the object, and help to eliminate any of the ultra-violet which is deflected toward the objective.

§ **309. Immersion media.** — Non-fluorescing liquids must be used or the objects will appear in a bright fog. Fortunately for an immersion liquid there is available the medicinal mineral oil (petrolatum, nujol, etc.). Both those with a paraffin and those with a naphthalene base are wholly non-fluorescing for the ultra-violet most useful for microscopy (fig. 129), that is, the ultra-violet from the mercury or carbon arc which is transmitted through the screens used to eliminate the visible light.

Fortunately also, the water in which the small organisms live is non-fluorescing, and the isotonic solutions used in physiological experiments are likewise non-fluorescing, so that all desired physiological tests can be made with living things and living tissues.

§ **310. Permanent mounting media.** — Canada balsam is fluorescing and therefore not available. The medicinal mineral oil answers very well, but the cover-glass must be sealed with shellac or other sealing cement. The steps for permanent preparations are exactly as for balsam mounting (§§ 534–535) except that the mineral oil is used instead of the balsam. Its index of refraction (nD 1.48) is almost as great as Canada balsam and cedar oil, so that it can be substituted with little loss in optical results.

§ **311. Ultra-violet lamp.** — The recently developed 100-watt H4 capillary arc (fig. 129 *A*) is the most satisfactory source of ultra-violet for the microscope and for photomicrography (see § 470). It is also good for naked-eye fluorescence phenomena shown by chlorophyll solutions, colored cloth and masses of any substance (see p. 257).

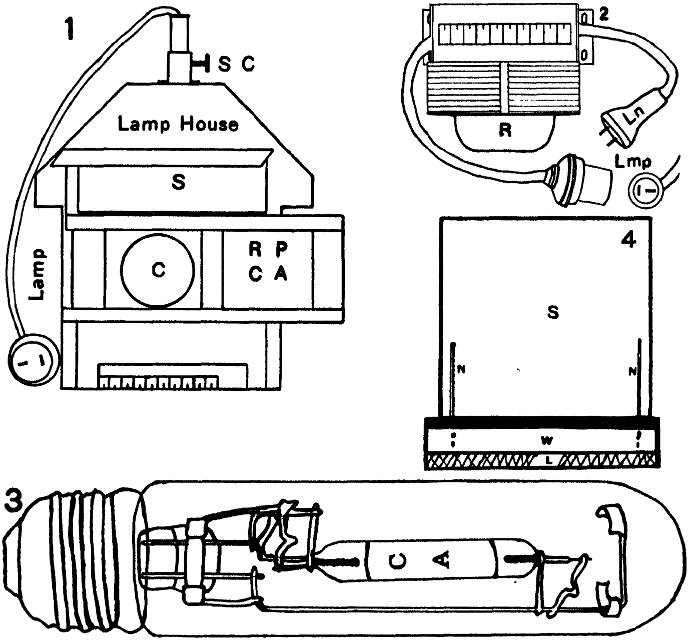

Fig. 129 *A*. Capillary Mercury Arc Lamp for Fluorescence and for Photomicrography.

(*1*) *Lamp House* The metal container of the capillary mercury arc bulb (*3*).

SC Screw for holding the tube containing the electric cable to the socket. It serves to regulate the height of the mercury arc. This is in a tube fastened to a metal plate that can be moved toward or from the front of the lamp house, to change the position of the mercury arc.

Lamp This word is placed just above the polarized socket of the cable going to the lamp socket.

S This is placed under the middle of the sloping screen to shut off the radiation which would extend upwards to the eyes of the observer.

C Free opening of the lamp house when both visible and invisible radiation is desired.

RP CA Red-Purple Corex A filter to transmit radiations between 250 $m\mu$

and 410 mμ. The clear opening and the ultra-violet filter are in a sliding carrier so that either may be used at will. The actual size is indicated by the 10-cm. scale at the bottom of the lamp house.

(2) Alternating current regulator for the capillary arc.

Ln Plug for connecting with the line.

Lamp Polarized plug for connecting with the cable to the lamp.

R Part of the regulator.

The oval openings on the two sides of the electric cables near the top are for screws to hold the regulator in place. For the inexperienced, it would be wise to have the manufacturers add the cables with plug and socket to the regulator.

(3) Transparent view of the capillary mercury arc bulb with its glass case.

CA Capillary arc within its glass sheath. (Two-thirds natural size.)

(4) Block for holding the filter in photomicrography.

S Screen or filter 5 to 7.5 cm. square.

n–n Two of the four headless nails to hold the screen upright.

W Wooden block and (*L*) lead on the bottom.

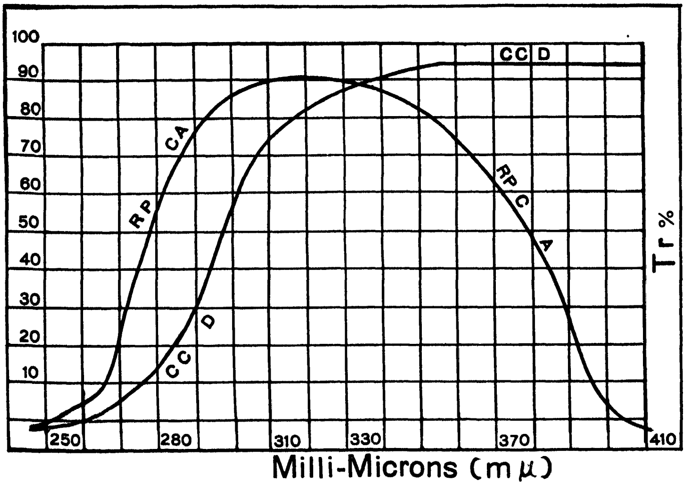

FIG. 129 *B*. SIMPLE CURVE TO SHOW THE TRANSMISSION OF ULTRA-VIOLET AND VISIBLE RADIATION OF THE CORNING CLEAR COREX D GLASS AND OF THE RED-PURPLE COREX A GLASS.

In the curves the base-line or abscissa shows the wave length in millimicrons, and the vertical line the transmission percentage for the different wave lengths of the radiation.

RP CA. Red-Purple Corex A. It shows transmission between 250 mμ and 410 mμ, the maximum being between 280 mμ and 370 mμ. As there is some transmission from 390–410 mμ, there is a limited amount of visible radiation in this filter.

CC D. Clear Corex D. This curve also shows transmission commencing at 250 mμ with the maximum ultra-violet at 360 mμ. From that point onward there is between 90 and 100% transmission.

It takes about five minutes for the lamp to emit the full amount of ultra-violet. In working with the high pressure mercury arc one should wear goggles with glass opaque to ultra-violet, or should be careful not to look at the full light. The eyes might be injured. The crystalline lens of the eye is highly fluorescent. If much ultra-violet got into the eyes everything would appear as in a bright fog.

§ **314. Spectroscope used with the ultra-violet microscope.** — This enables one to determine the wave lengths of visible light present in the fluorescent radiation from the object. Sometimes all the colors of the rainbow are represented. As the fluorescent light is rarely pure white, some of the colors will be more intense than others. Sometimes only a part of the colors are represented, and in some cases there will be absorption bands. (See Fluorescence of the Uranyl Salts by Nichols, pp. 120–121.) In case one does not possess a micro-spectroscope, a pocket spectroscope (§ 293a) will answer fairly well.

To make sure that all the light passing through the spectroscope comes from a definitely fluorescing object, one must be careful to have the object limited by the slit, or be the only object lighted (§§ 291, 293a).

EXAMPLES OF THE USE OF THE ULTRA-VIOLET MICROSCOPE

§ **315. Anthracene crystals.** — This is an example of mixed crystals, both of which are highly fluorescent. It illustrates the manner of arranging the microscope on the one hand, and of detecting mixtures on the other.

The room is darkened, or one works at night. The mercury arc lamp is lighted (§ 313), and the microscope put opposite the lamp-house window at about 22 cm. distance. The quartz bull's-eye condenser is placed at about 8 to 10 cm. from the lamp window. To determine quickly and certainly when the microscope, the bull's-eye condenser and the radiation from the lamp are in line and properly arranged, one can use a card smeared with anthracene in a strong solution of cane sugar. This fluoresces so brilliantly that one can adjust the different elements accurately. The ultra-violet beam should be focused on the specimen. At first one can focus the light on the reflector. Then to get the beam up through the condenser to the specimen, one can use a corex slip mount of the anthracene in sugar. When the radiation reaches it, it will fluoresce. Then by looking through the microscope with a 16 mm. (10x) objective and a 5x or other low ocular in place, focus the crystals, and by adjusting the prism or the bull's-eye quartz condenser or both, get the brightest light possible. This is the procedure up to the present for all specimens, the anthracene slide acting as a guide or indicator. For the actual study of any specimen, the under side of the corex slip should be in immersion contact with the top of the condenser, using the non-fluorescing immersion petrolatum, (§ 309).

If one studies the anthracene preparation, a part of the crystals will fluoresce blue, and a part yellow-green. The yellow-green crystals are in excess of the blue ones. The blue crystals represent the pure anthracene, and the yellow-green ones contain chrysogen (Nichols and Howe, Fluorescence of the Uranyl Salts, p. 12). By moving the screen carrier, one can see the appearance in the full mercury arc radiation.

§ 316. Elastic tissue, physical analysis. — This tissue is abundantly present in all adult vertebrates with the possible exception of three of the lowest forms: Amphioxus, myxine and the lamprey. It is present in increasing quantity as the animal series advance in the zoölogical scale until finally in adult man almost all of the connective substance in the body contains a greater or less amount of elastic tissue.

It is relatively late in appearance in the embryo, and steadily increases in amount with the added years. Like all the other tissues of the body, it is surrounded by and in a kind of matrix of collagenous or white fibrous connective tissue. It is easily obtained in a nearly pure form in the elastic ligament of the neck (ligamentum nuchæ) in grazing animals. The only drawback of this tissue for illustrating the advantages of physical analysis is that there is no striking difference in appearance to the naked eye, nor in its reactions when alive and when dead.

§ **317. Fresh material.** — A piece of the ligamentum nuchæ of a recently killed beef animal is secured and its satiny appearance noted by the naked eye.

With a sharp razor or safety razor blade wet with normal salt solution, make as thin a section as possible free-hand across the end of the ligament or a part of it. Place on a corex slip and add a drop of normal salt solution. Cover with an ordinary cover-glass. Make a similar section lengthwise of the ligament, and mount on a corex slip as before.

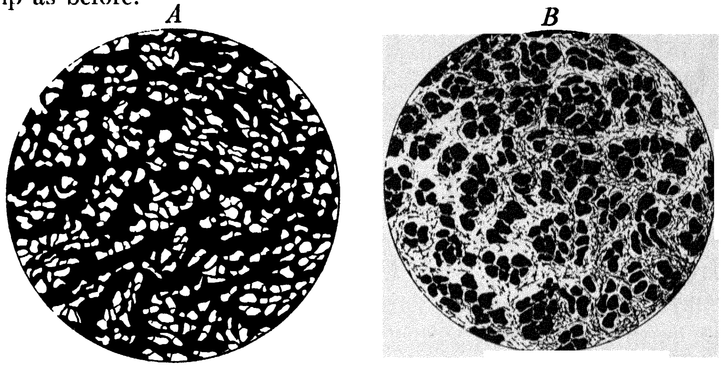

FIG. 130 *A*, *B*. SKETCHES TO SHOW THE APPEARANCE OF A CROSS SECTION OF ELASTIC TISSUE: *A*. UNDER THE ULTRA-VIOLET MICROSCOPE. *B*. UNDER THE DARK-FIELD MICROSCOPE.

They look like positive and negative images of the same thing. Compare with C, D.

If the mercury arc has been lighted for five minutes or more so that the full amount of ultra-violet is being given off, hold the piece of ligamentum nuchæ in the path of the ultra-violet. It will fluoresce with a white light slightly tinged with blue. The appearance is striking and, when once seen, will not be forgotten. The sections will also fluoresce so that they stand out on the corex slips.

§ **318. Microscopic examination of the fresh sections.** — Making sure that the ultra-violet is passing up through the quartz condenser by the use of an anthracene specimen (§ 315), put the cross section of the elastic tissue in place and in immersion contact with the top of the condenser. Use first a 16 mm. (10x) objective, and later an 8 mm. (20x) objective or a higher one. When the microscope is in focus, it will be seen that the cut ends of the elastic fibers glow with a soft bluish-white radiance. Between the cut ends the specimen will appear dark. Note carefully the location of one of the light areas, or set the pointer of the ocular (fig. 40) upon it. Then move the screen carrier along until the visible light from the mercury lamp passes to the object. One will get a dark-field image, and what was dark with the ultra-violet radiation will appear brilliantly light in the dark-field picture. The elastic fibers by contrast will appear dark. That is, the two appearances are the positive and the negative images of each other (fig. 130 A, B).

Remove the cross section, and put under the microscope the longitudinal section of the elastic tissue. Use the ultra-violet and the visible light of the mercury arc by moving the ultra-violet filter in place and then the corex glass. Here the elastic fibers will be seen in their length, and will fluoresce just as did the cross sections. The ordinary connective tissue will also behave as in the cross section.

§ **319. For the spectral colors.** — Make sure that the fluorescent light is as brilliant as possible with the ultra-violet filter in place. Remove the ocular and put in its stead the spectroscope. This will show the colors making up the fluorescent light. The spectrum will show all the colors, but the brightest part will be in the blue-green.

§ **320. Elastic tissue with the polarizing microscope.** — Use the same preparations as for the ultra-violet experiment. Put them under the polarizing microscope, and cross the nicols. The elastic tissue does not polarize under ordinary conditions, therefore it will remain dark with crossed nicols. The white fibrous tissue does polarize, that is, is anisotropic when the fibers are at right angles to the axis of the microscope, but not when seen in cross section or in oblique positions. Then the fibers will remain dark with crossed nicols.

Comparing the polariscopic picture with the dark-field appearance, it will be seen that the dark areas are relatively very large and the bright part very small. This is due to the fact that most of the fibers of the ordinary connective tissue are not at right angles to the microscope axis, and hence join the elastic tissue in producing dark areas (fig. 130, B, C).

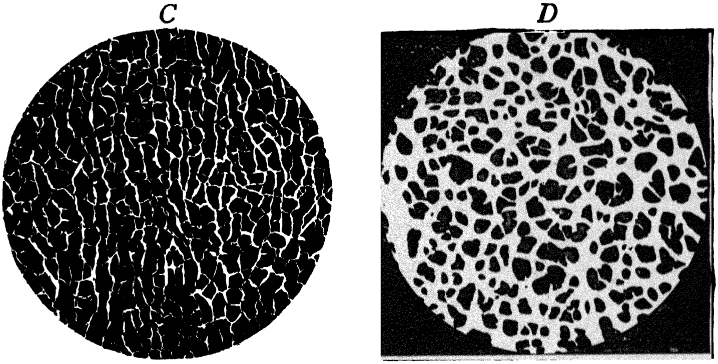

FIG. 130. *C D* SKETCHES TO SHOW THE APPEARANCE OF A CROSS SECTION OF ELASTIC TISSUE: *C* UNDER THE POLARIZING MICROSCOPE, *D*. STAINED AND UNDER THE BRIGHT-FIELD MICROSCOPE.

In *C* the connective tissue with fibers at right angles to the axis of the microscope polarize light while the elastic tissue and the connective tissue not at right angles to the microscope axis remain dark. Compare with the true amount of ordinary connective tissue shown light in *B* and dark in *D*.

In *D*. the ordinary connective tissue was stained blue and the elastic tissue pink with Mallory's connective tissue stain.

§ **321. Fixed Elastic Tissue with the ultra-violet microscope.** — It was found that elastic tissue fixed two or three days in a mixture of Mueller's fluid and formalin (Mueller's 90 cc., strong formalin 10 cc.), washed in water for half a day and then imbedded in paraffin in the usual manner, and sectioned with a microtome, gave all the reactions shown by the fresh material. The sections should be thin, 5μ to 7μ. No albumen fixative should be put on the slide for it fluoresces. The slips for mounting should be of corex or quartz. After the sections are dry on the slip, the paraffin is removed by xylene and the sections covered with an ordinary cover-glass on which is a large drop of the petrolatum. Seal the cover with shellac. Examine exactly as for the fresh material, both with the ultra-violet and the polarized light. The appearances are practically the same as for the fresh material.

PHYSICAL ANALYSIS OF STRUCTURE

In Ultra-Violet and in Visible Radiation

COMPARISON WITH STAINING

DATE and NAME

Naked-Eye Appearance	Living	
	Fresh	
	Fixed	
Ultra-Violet Microscope with Corex Glass Filter and Dark-Field	Living	
	Fresh	
	Fixed	
Micro-Spectroscope For Colors in Fluorescence	Living	
	Fresh	
	Fixed	
Polarizing Microscope	Living	
	Fresh	
	Fixed	
Micro-Incineration for Mineral Contents	Fixed	
Vital and Other Staining In Comparison with Physical analysis		

§ 322. Comparison by different methods. — It is of great interest to compare physical appearances under the polarizing and ultra-violet microscope with neighboring sections of the same tissue stained in various ways; also of incinerated specimens. Such a comparison gives one a conception of how complex are the structures of the body, and how limited is the information gained by any single method of treatment (see fig. 130 *AB*, *CL*).

For the staining see under elastic stains in § 582.

For the orcein stain, counterstain with methylene blue to bring out the nuclei, and note that these are confined to the ordinary connective tissue, none being found in the elastic tissue.

Ultra-Violet with Microscopic Animals. Ciliated Epithelium, etc.

§ **323. Minute animals and ultra-violet.** — To test the effect of ultra-violet on minute animal life, make a preparation of the living forms found in an infusion (§§ 210, 543). Use corex for a slip, and the water in which the animals naturally live for a normal mounting medium. Make immersion contact with the condenser and allow the ultra-violet passing through the red-purple corex (fig. 129) to act on the organisms for, say five minutes, then examine them with the visible light of the daylight lamp, (fig. 79, or 80). Have a similar preparation under the usual dark-field microscope as control. Compare the appearance of the two preparations. Continue the short exposures to ultra-violet and find out how long the animals live, and what changes take place. To prevent the drying out of the mounting liquid, seal the cover with oil as for a fresh blood preparation (§ 211). Do the same for the control.

For a preparation of ciliated cells, scrape the roof of the throat of a live frog. Mount on a corex slip using aqueous humor, or the blood of the frog for a mounting medium. Seal the cover as for blood. Prepare a control in the same way. Expose to ultra-violet as directed for the minute animals, and note any effect.

Green Plant Tissue under the Ultra-Violet Microscope

§ **324. Fluorescence of plant structures.** — The fact that the green substance of plants fluoresces red was discovered by Sir David Brewster in 1833. (See Stokes, Philos. Trans., Vol. 142, pp. 463–464.) Brewster used a solution of chlorophyll in alcohol, and that is still the ordinary method of showing the amazing change in color when chlorophyll is submitted to ultra-violet radiation.

It is not necessary to put the chlorophyll in solution to get the red fluorescence. This was strikingly shown by the chloroplastids obtained from the leaves of the snapdragon (antirrhinum) which had become macerated in a flower vase. Following this hint, sections were made free-hand of the green leaves of many different plants, mounted on corex slips in water, and examined under the ultra-violet microscope. All of them showed the red chlorophyll bodies. It was found later that the easiest and most effective way to get sections of the most favorable thickness, and to secure isolated chloroplastids, was to put the blade of grass or the other chlorophyll-bearing structure on a corex slip in some water and scrape it with a moderately sharp scalpel or other knife. The fragments thus obtained show everything. One of the most strikingly beautiful and instructive preparations was made in this way by placing a blade of grass on a corex slip and scraping it. Some of the fragments showed the individual chloroplastids in their cells. Other chloroplastids were free. All fluoresced a beautiful red. The cellulose veins extend lengthwise, and fluoresce a brilliant bluish white. The appearance was then like a ribbon with brilliant, narrow, white stripes, and broad red ones.

Under the polarizing microscope with crossed nicols the cellulose glowed with a brilliant white, but the chloroplastids did not polarize. However, the light given off by the cellulose veins and the cell walls of the plastids and the parenchyma of the tissue is enough to bring out the green color of the chlorophyll. Occasionally in these scraped preparations some of the chloroplastids are isolated, and if one is studied with a high power, it will be seen that the wall polarizes, and that the chlorophyll is green even with crossed nicols.

It is believed that the physical analysis by means of the ultra-violet microscope, the polarizing and the dark-field microscope will prove of as much help to the botanist as to the animal histologist.

ULTRA-VIOLET FOR NAKED EYE DEMONSTRATIONS

Unless one has paid attention to such matters, it is unbelievable that the same object under different kinds of light or radiation should appear so strikingly different. For a good example, take a cheap, red bandanna handkerchief. Look at it by daylight, by kerosene light, and by the ordinary mazda light. Then hold it in the beam of ultra-violet, next in the visible mercury light. Chlorophyll has already been cited. Quinine in water does not show at all in daylight, but in the ultra-violet it glows with a wonderful blue-white radiance.

Figured dress goods, cheap, brightly colored handkerchiefs, neckties, etc., give a change in appearance which is truly marvelous.

These naked-eye appearances with different radiation make the thoughtful person appreciate how many facts must be taken into consideration in order to gain a true conception of the appearance of what seem the simplest things in nature and art. After such an exhibition one feels deeply the need of caution in one's statements, and the danger of being dogmatic about anything, unless all the conditions and the circumstances are thoroughly understood.

COLLATERAL READING

In the first place should be mentioned the fundamental contribution of George Gabriel Stokes. Philos. Trans. of the Royal Society, Vol. 142, p. 555 et. sq. Change in the Refrangibility of Light. On p. 479 in a note at the bottom he says: "I do not like this term (David Brewster's *Internal Dispersion*) and am almost inclined to coin a word and call the appearance *Fluorescence*, from fluor-spar as the analagous *opalescence* is derived from the name of a mineral." This term has received universal approval.

On p. 503 in describing fluorescence he says: "We may express the result extremely well by saying that the fluid or solid medium (which fluoresces) is self-luminous so long as it is under the influence of the active light."

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CHAPTER VII

INTERPRETATION OF APPEARANCES

§§ 325–358; FIGURES 131–142

§ **325. Appearances** which seem perfectly unmistakable with a low power may be found erroneous or very inadequate with high powers; for details of structure which cannot be seen with a low power may become perfectly evident with a higher power or a more perfect objective. On the other hand, the problems of microscopic structure become more and more complex with increased precision of investigation and more perfect optical appliances, for structures that appeared intelligible with a less perfect microscope may show complexities in their details of structure with the more perfect microscope which open up an entirely new field for interpretation. Further, if the specimen is viewed with the dark-field microscope, the polarizing and the ultra-violet microscope, wholly new appearances are almost sure to arise (§ 357, Ch. III, IV, V, VI).

One must always be on the lookout for errors in judgment induced by color effects due to purely optical means and to color in the specimen, and also to avoid confusing refraction, reflection, and diffraction effects with pigments, or actual structures of any kind. It is not infrequent in searching for malarial pigment in the red blood corpuscles to mistake the dark-looking crenations on the corpuscles for the pigment sought (§ 326).

The need of the most careful observation and constant watchfulness lest the appearances may be deceptive is thus admirably stated by Dallinger. (See Carpenter-Dallinger, p. 427): " The correctness of the conclusions which the microscopist will draw regarding the nature of any object from the visual appearances which it presents to him when examined in the various modes now specified will necessarily depend in a great degree upon his previous experience in microscopic observation and upon his knowledge of the class of bodies to which the particular specimen may belong. Not only are observations of *any* kind liable to certain fallacies arising out of the previous notions which the observer may entertain in regard to the constitution of the objects or the nature of the actions to which his attention is directed, but even the most practised observer is apt to take no note of such phenomena as his mind is not prepared to appreciate. Errors and imperfections of this kind can only be corrected, it is obvious, by general advance in scientific knowledge; but the history of them affords a useful warning against hasty conclusions drawn from a too cursory examination. If the history of almost any scientific investigation were fully made known, it would generally appear that the stability and completeness of the conclusions finally arrived at had been only attained after many modifications, or even entire alterations of doctrine. And it is therefore of such great importance as to be almost essential to the correctness of our conclusions that they should not be finally formed and announced until they have been tested in every conceivable mode. It is due to science that it should be burdened with as few false facts [artifacts] and false doctrines as possible. It is due to other truth-seekers that they should not be misled, to the great waste of their time and pains, by our errors. And it is due to ourselves that we should not commit our reputation to the chance of impairment by the premature formation and publication of conclusions which may be at once reversed by other observers better informed than ourselves, or may be proved fallacious at some future time, perhaps even by our own more extended and careful researches. *The suspension of the judgment whenever there seems room for doubt* is a lesson inculcated by all those philosophers who have gained the highest repute for practical wisdom; and it is one which the microscopist cannot too soon learn or too constantly practise."

The general law for the whole matter is to study the object in every way possible (§ 358).

For the experiments, §§ 327–340, *no condenser is to be used, except in a part of* § 340.

§ **326.** "The distinction between a dark element which is referable to pigment and a dark element which is referable to the deflection of light can generally be made by watching the effect produced by the alteration of the focus. Where the dark element corresponds to a point from which light is deflected a change of the focus will be associated with a change from dark to bright. Where pigment is in question a change of focus will substitute only a more diffuse for a less diffuse dark element." (Wright, p. 44.)

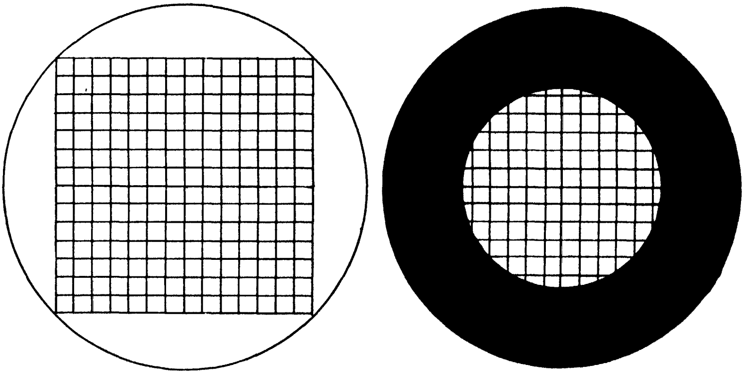
§ 327. Dust or Cloudiness on the Ocular. — Employ the 16 mm. 10x objective, 4x or 5x ocular, and fly's wing as object.

Unscrew the field lens and put some particles of lint from dark cloth on its upper surface. Replace the field lens and put the ocular in position (§ 85). Light the field well and focus sharply. The image will be clear, but part of the field will be obscured by the irregular outline of the particles of lint. Move the object to make sure this appearance is not due to it.

Grasp the ocular by the milled ring just above the tube of the microscope and rotate it. The irregular objects will rotate with the ocular. Cloudiness or particles of dust on any part of the ocular may be detected in this way.

Unscrew the field lens and remove the lint before proceeding.

§ 328. A small bright field. — With low objectives (25–50 mm. [5x–3.2x]) if too small a diaphragm is used and put close to the object, only the central part of the field will be illuminated, and around the small light circle will be seen a dark ring (fig. 132). If



FIGS. 131, 132. THE MICROSCOPIC FIELD COMPLETELY AND ONLY PARTLY ILLUMINATED.

A The field completely illuminated; a net micrometer is used as object.

B The field is only partly illuminated; the same net micrometer is used as object, but not all of it appears in the partially lighted field.

the diaphragm is lowered or a sufficiently large one employed, the entire field will be lighted (fig. 131). (See also § 131 for diaphragms with the condenser).

§ 329. Relative position of objects or parts of the same object. — The general rule is that objects highest up come into focus last in focusing up, first in focusing down.

§ 330. Objects having plane or irregular outlines. — As object use three printed letters in stairs mounted in Canada balsam (fig. 133). The first letter is placed directly upon the slide, and covered with a small piece of glass about as thick as a slide. The second letter is placed upon this and covered in like manner. The third letter is placed upon the second thick cover and covered with an ordinary cover-glass. The letters should be as near together as possible, but not overlapping. Employ the same ocular and objective as above (§ 327).

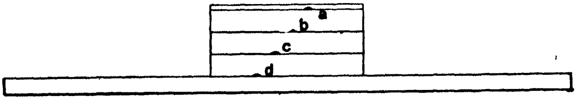


FIG. 133. LETTERS IN STAIRS TO DETERMINE RELATIVE POSITION BY FOCUSING UP AND DOWN.

Lower the tube till the objective almost touches the top letter; then look into the microscope and slowly focus up. The lowest letter will first appear and then, as it disappears, the middle one will appear and so on. Focus down, and the top letter will first appear, then the middle one, etc. The relative position of objects is determined exactly in this way in practical work.

For example, if one has a micrometer ruled on a cover-glass 0.15–0.25 mm. thick, it is not easy to determine with the naked eye which is the ruled surface. But if one puts the micrometer under a microscope and uses a 4 mm. (40x) objective, it is easily determined. The cover should be laid on a slide and focused till the lines are sharp. Now, without changing the focus in the least, turn the cover over. If it is necessary to focus up to get the lines of the micrometer sharp, the lines are on the upper side. If one must focus down, the lines are on the under surface. With a thin cover and delicate lines this method of determining the position of the rulings is of considerable practical importance.

§ **331. Determination of the form of objects.** — The procedure is exactly as for the determination of the form of large objects. That is, one must examine the various aspects. For example, if one were placed in front of a wall of some kind, one could not tell whether it was a simple wall or whether it was one side of a building unless in some way one could see more than the face of the wall. In other words, in order to get a correct notion of any body, one must examine more than one dimension, — two for plane surfaces, three for solids. So for microscopic objects, one must in some way examine more than one face. To do this with small bodies in a liquid the bodies may be made to roll over by pressing on one edge of the cover-glass. And in rolling over the various aspects are presented to the observer. With solid bodies, like the various organs, correct notions of the form of the elements can be determined by studying sections cut at right angles to each other. The methods of getting the elements to roll over, and of sectioning in different planes, are in constant use in histology, and the microscopist who neglects to see all sides of the tissue elements has a very inadequate and often a very erroneous conception of their true form.

§ **332. Transparent objects having curved outlines.** — The success of these experiments will depend entirely upon the care and skill used in preparing the objects in lighting and in focusing.

Employ a 4 mm. (40x) or higher objective and a 10x ocular for all the experiments. It may be necessary to shade the object (§ 155) to get satisfactory results. When a diaphragm is used, the opening should be small and it should be close to the object.

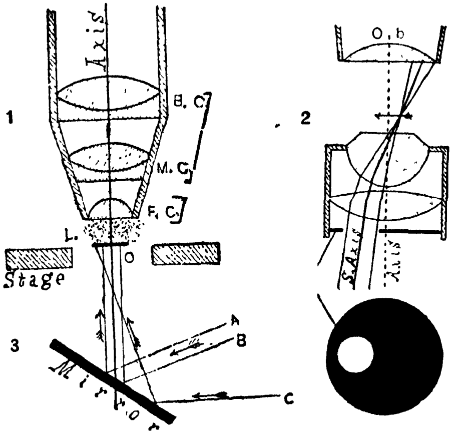
§ **333. Air bubbles.** — Prepare these by placing a drop of thin gum arabic mucilage on the center of a slide and beating it with a scalpel blade until the mucilage looks milky from the inclusion of air bubbles. Put on a cover-glass but do not press it down.

§ **334. Air bubbles with central illumination.** — Shade the object, and with the plane mirror light the field with central light (fig. 20).

Search the preparation until an air bubble is found appearing about 1 mm. in diameter, get it into the center of the field, and if the light is central the air bubble will appear with a wide, dark, circular margin and a small, bright center. If the bright spot is not in the center, adjust the mirror until it is.

This is the simplest and surest method of telling when the light is central or axial when no condenser is used (§ 110).

Focus both up and down, noting that, in focusing up, the central spot becomes very clear and the black ring very sharp. On elevating the tube of the microscope still more, the center becomes dim, and the whole bubble loses its sharpness of outline.



Figs. 134–135. Oblique Illumination with a Mirror and with a Condenser.

1 The light is shown to be oblique with ray *c*; rays *A B* are central. The arrows indicate the path of the rays. (For the objective see explanation of figure 44.)

2 Abbe condenser with an eccentric diaphragm (*3*) admitting light only on one side.

Axis The principal optic axis. *Ob* Objective.

S Axis Secondary axis.

§ **335. Air bubbles with oblique illumination.** — Remove the substage of the microscope and all the diaphragms. Swing the mirror so that the rays may be sent very obliquely upon the object (fig. 134). The bright spot will appear no longer in the center, but on the side away from the mirror (fig. 136 *A*).

§ **336. Oil globules.** — Prepare these by beating a small drop of clove or other oil with gum arabic mucilage on a slide and covering as directed for air bubbles (§ 333), or use a drop of milk in a drop of water.

§ **337. Oil globules with central illumination.** — Use the same diaphragm and light as above (§ 334). Find an oil globule appearing about 1 mm. in diameter. If the light is central, a bright spot will appear in the center. Focus up and down and note that the dark ring is narrower than with air and that the bright center of the oil globule is clearest last in focusing up.

§ **338. Oil globules with oblique illumination.** — Remove the substage, etc., as above, swing the mirror to one side and light with oblique light. The bright spot will be eccentric, and will appear to be on the same side as the mirror (fig. 136).

§ **339. Oil and air together.** — Make a preparation exactly as described for air bubbles (§ 333), and add at one edge a little of the mixture of oil and mucilage (§ 336); cover and examine.

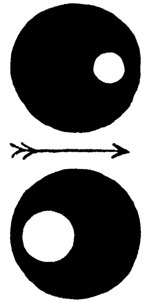


FIG. 136. SMALL AIR BUBBLE (*A*) AND OIL GLOBULE (*O*) WITH OBLIQUE LIGHT.

The arrow indicates the direction of the light.

The substage need not be used in this experiment. Search the preparation until an air bubble and an oil globule, each appearing about 1 mm. in diameter, are found in the same field of view. Light first with central light, and note that, in focusing up, the air bubble comes into focus first and that the central spot is smaller than that of the oil globule. Then, of course, the black ring will be wider in the air bubble than in the oil globule. Make the light oblique. The bright spot in the air bubble will move away from the mirror, while that in the oil globule will move toward it (fig. 136).

As the air bubble is of less refractive index than the mucilage, it will act like a concave lens (fig. 137), while the oil globule, having a greater refractive index than the mucilage, will act as a convex lens (fig. 137, § 339a).

It is possible to distinguish oil and air optically, as described above, only when quite high powers are used and very small bubbles are selected for observation. If a 16 mm. (10x) objective is used instead of a 4 mm. (40x), the appearances will vary considerably from that given above for the higher power. It is well to use a low as well as a high power. Marked differences will also be seen in the appearances with objectives of small and of large aperture, as the larger aperture takes in more oblique rays and hence the black margin is narrowed (§ 341).

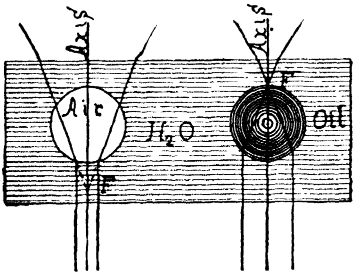


FIG. 137. AIR BUBBLES AND OIL GLOBULE IN WATER.

Axis The principal optic axis.
F, *F* The principal foci of the air and oil. As the air is less refractive than water its focus is virtual. The focus of the oil globule is real, as its refraction is greater than water.

§ **339a.** It should be remembered that the image in the compound microscope is inverted (fig. 18); hence the bright spot really moves toward the mirror for air, and away from it for oil.

§ **340. Air and oil by reflected light.** — Use the same preparation as in § 339. Cover the diaphragm or mirror so that no transmitted light can reach the preparation. The oil and air will appear like globules of silver on a dark ground. The part that was darkest in each with transmitted light will be lighted, and the bright central spot will be somewhat dark. Use also the condenser and dark-ground illumination.

Experiments in which the substage condenser is used (§§ 341–348).

§ **341. Distinctness of outline.** — In refraction images this depends on the difference between the refractive power of a body and that of the medium which surrounds it. The oil and air were very distinct in outline, as both differ greatly in refractive power from the medium which surrounds them, the oil being more refractive than the mucilage and the air less (fig. 137).

Place a fragment of a cover-glass on a clean slide, and cover it (fig. 138). Use it as object and employ the 16 mm. (10x) objective and 8x or 10x ocular. The fragment will be outlined by a dark band. Put a drop of water at the edge of the cover-glass. It will run in and immerse the fragment. The outline will remain distinct, but the dark band will be somewhat narrower. Remove the cover-glass, wipe it dry, and wipe the fragment and slide dry also. Put a drop of 50% glycerin on the middle of the slide and mount the fragment of cover-glass in that. The dark contour will be much narrower than before.

Draw a solid glass rod out to a fine thread. Mount one piece in air, and the other in 50% glycerin. Put a cover-glass on each. Employ the same optical arrangement as before. Examine the one in air first. There will be seen a narrow, bright band, with a wide, dark band on each side (fig. 139a).

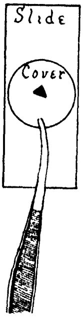


FIG. 138. FINE FORCEPS FOR PLACING COVER-GLASSES ON SPECIMENS.

The one in glycerin will show a much wider bright central band, with the dark borders correspondingly narrow (fig. 139b). The dark contour depends also on the numerical aperture of the objective — being wider with low apertures. This can be readily understood when it is remembered that the greater the aperture the more oblique the rays of light that can be received, and that the dark band simply represents an area in which the rays are so greatly bent or refracted (fig. 137) that they cannot enter the objective and contribute to the formation of the image; the edges are dark simply because no light from them reaches the observer.

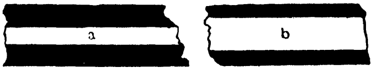


FIG. 139. GLASS RODS IN AIR AND IN GLYCERIN.

a Glass rod in air and viewed by central transmitted light.

b Glass rod mounted in 50% glycerin; the dark border is narrower than when mounted in air.

If the glass rod or any other object were mounted in a medium of the same color and refractive power, it could not be distinguished from the medium.

The effect of the immersing liquid on the contour bands around any transparent object is made of practical use in the determination of the refractive index of crystals and other bodies. When the crystal and liquid are of the same index there will be no band, and the more they differ, the wider will be the band. As shown in §§ 333–340, lighting with oblique light, also focusing up and down, will indicate whether the crystal is of greater or less index than the liquid. For this method a series of liquids of known index of refraction must be at hand. For a complete discussion, see Chamot, p. 185, Chamot and Mason, vol. I, p. 366.

A very striking and satisfactory demonstration may be made by painting a zone or band of eosin or other transparent color on a solid glass rod, and immersing the rod in a test tube or vial of cedar oil, clove oil, or turpentine. Above the liquid the glass rod is very evident, but under the liquid it can hardly be seen except where the red band is painted on it. This is a good example of a color image and of a refraction image to the naked eye (§ 152).

§ **341a.** Some of the rods have air bubbles in them, and then there results a capillary tube when they are drawn out. It is well to draw out a glass tube into a fine thread and examine it as described. The central cavity makes the experiment much more complex.

§ **342. Highly refractive.** — This expression is often used in describing microscopic objects (medullated nerve fibers, for example), and means that the object will appear to be bordered by a wide, dark margin when it is viewed by transmitted light. And from the above (§ 341), it would be known that the refractive power of the object and the medium in which it was mounted must differ considerably.

§ **343. Doubly contoured.** — This means that the object is bounded by two usually parallel, dark lines with a lighter band between them. In other words, the object is bordered by (1) a dark line, (2) a light band, and (3) a second dark line.

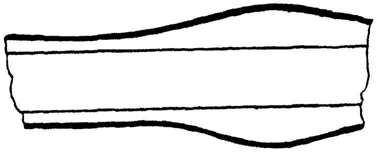


FIG. 140. SOLID GLASS ROD COATED WITH COLLODION TO SHOW DOUBLE CONTOUR.

This may be demonstrated by coating a fine glass rod (§ 341) with one or more coats of collodion or celloidin and allowing it to dry, and then mounting in 50% glycerin as above (§ 341). Employ a 4 mm. (40x) or higher objective, light with transmitted light, and it will be seen that where the glycerin touches the collodion coating there is a dark line, next this is a light band, and finally there is a second dark line where the collodion is in contact with the glass rod (fig. 140).

§ **343a.** The collodion used is a 6% solution of soluble cotton (parlodion, collodion, pyroxylin) in equal parts of sulphuric ether and 95%, or, preferably, absolute alcohol. It is well to dip the rod two or three times in the collodion and to hold it vertically while drying. The collodion will gather in drops, and one will see the difference between a thick and a thin membranous covering (fig. 140).

§ **344. Optical section.** — This is the appearance obtained in examining transparent or nearly transparent objects with a microscope when some plane below the upper surface of the object is in focus. The upper part of the object, which is out of focus, obscures the image but slightly. By changing the position of the objective or object, a different plane will be in focus and a different optical section obtained. The most satisfactory optical sections are obtained with high objectives having large aperture.

Nearly all the transparent objects studied may be viewed in optical section. A striking example will be found in studying mammalian red blood corpuscles on edge. The experiments with the solid glass rods (fig. 139) furnish excellent and striking examples of optical sections.

§ **345. Currents in liquids.** — Employ a 16 mm. (10x) objective, and as object put a few particles of carmine, starch, or chalk dust on the middle of a slide and add a drop of water. Grind the carmine or other substance well with a scalpel blade; leave the preparation uncovered. If the microscope is inclined, a current will be produced in the water, and the particles will be carried along by it. Note that the particles seem to flow up instead of down; why is this? How would it appear to flow with an erecting microscope?

§ **346. Velocity under the microscope.** — In studying currents or the movement of living things under the microscope, one should not forget that the apparent velocity is as unlike the real velocity as the apparent size is unlike the real size. If one consults (fig. 51, it will be seen that the actual size of the field of the microscope with the different objectives and oculars is inversely as the magnification. That is, with great magnification only a small area can be seen. The field appears to be large, however, and if any object moves across the field, it may appear to move with great rapidity, whereas if one measures the actual distance passed and notes the time, it will be seen that the actual motion is quite slow. One should keep this in mind in studying the circulation of the blood. The truth of what has just been said can be easily demonstrated in studying the circulation in the gills of necturus, or in the frog's foot, by using first a low power in which the field is actually of considerable diameter (fig. 51; Table, § 94) and then using a high power. With the high power the apparent motion will seem much more rapid. For spiral, serpentine, and other forms of motion, see Carpenter-Dallinger, p. 433.

§ **347. Pedesis or Brownian movement.** — Employ the same object as above, but a 4 mm. (40x) or higher objective in place of the 16 mm. (10x). Make the body of the microscope vertical so that there may be no currents produced. Use a small diaphragm and light the field well. Focus and there will be seen in the field large motionless masses, and between them small masses in constant motion. This is an indefinite, dancing, or oscillating motion.

This indefinite but continuous motion of small particles in a liquid is called Brownian movement or pedesis; also, but improperly, molecular movement, from the smallness of the particles.

The motion is increased by adding a little gum arabic solution or a slight amount of silicate of soda or soap; sulphuric acid and various saline compounds retard or check the motion. One of the best objects is lamp-black ground up in water with a little gum arabic. Carmine prepared in the same way, or simply in water, is excellent; and very finely powdered pumice-stone in water has for many years been a favorite object. Pedesis is exhibited by all solid matter if it is finely enough divided. For high powers, and with the dark-field microscope a very dilute mixture of carbon ink in water is excellent. For the dark-field microscope the chylomicrons of the blood show the Brownian movement admirably (§ 212).

Compare the pedetic motion with that of a current by slightly inclining the tube of the microscope. The small particles will continue their independent leaping movements while they are carried along by the current. The pedetic motion makes it difficult to obtain good photographs of milk globules and other small particles. The difficulty may be overcome by mixing the milk with a very weak solution of gelatin and allowing it to cool (10 % gelatin is good).

Until recently no adequate explanation of this movement had been offered. At the present time it is believed to be due to the kinetic activity of matter, and in itself to be one of the best proofs of that activity. This is what is said by Rutherford: "The character of the Brownian movement irresistibly impresses the observer with the idea that the particles are hurled hither and thither by the action of forces resident in the solution, and that these can only arise from the continuous and ceaseless movement of the invisible molecules of which the fluid is composed." "Whatever may be the exact explanation of this phenomenon, there can be but little doubt that it results from the movements of the molecules of the solution, and is thus a striking, if somewhat indirect, proof of the general correctness of the kinetic theory of matter." Nature, Vol. 81, 1909, pp. 257–263; Science, N. S., Vol. 30, 1909, pp. 289–303.

By the aid of the ultra-microscope it has been shown that the particles in smoke, etc., exhibit the pedetic movement even more strikingly than do those in liquids.

§ 348. Demonstration of pedesis with the polarizing microscope. — The following demonstration shows conclusively that the pedetic motion is real and not illusory (Ranvier, p. 173).

Open the abdomen of a dead frog (an alcoholic or formalin specimen is satisfactory). Turn the viscera to one side and observe the small whitish masses at the emergence of the spinal nerves. With fine forceps remove one of these and place it on the middle of a clean slide. Add a drop of water, or of water containing a little gum arabic. Rub the white mass around in the drop of liquid and soon the liquid will have a milky appearance. Remove the white mass, place a cover-glass on the milky liquid, and seal the cover by painting a ring of castor oil all around it, the ring being half on the slide and half on the cover-glass. This is to avoid the production of currents by evaporation.

Put the preparation under the microscope and examine with first a low power, then a high power (4 mm. 40x). In the field will be seen multitudes of crystals of carbonate of lime; the larger crystals are motionless, but the smallest ones exhibit marked pedetic movement.

Use the micro-polariscope, light with great care, and exclude all adventitious light from the microscope by shading the object (§ 155) and also by shading the eye. Focus sharply and observe the pedetic motion of the small particles, then cross the polarizer and analyzer, that is, turn one or the other till the field is dark. Part of the large motionless crystals will shine continuously and a part will remain dark, but small crystals between the large ones will shine for an instant, then disappear, only to appear again the next instant. This demonstration is believed to furnish absolute proof that the pedetic movement is real and not illusory.

For the help given by the micro-spectroscope see Ch. V.

§ 349. Use of dark-ground illumination for interpreting appearances. — Dark-ground illumination is almost invaluable for bringing out details of structure and for showing movement in living things. The granules and different parts in living cells and minute organisms are of so nearly the same refractive index that it is exceedingly difficult to differentiate them with the ordinary methods of illumination. On the other hand, with dark-ground illumination the different structures stand out with the greatest clearness.

§ 350. Specimens to use for dark-ground illumination. — (1) Organisms from hay infusion. Use for the infusion a small fruit jar or other glass dish. Go to a stream or pond and from a shallow, stagnant pool along the edge take some of the surface of the mud and put it into the jar with some of the water. Add some of the dead grass found along the edge of the pond; cut up into short pieces. Set in a warm, dimly lighted or dark place for a day or longer. This should soon be alive with all sorts of minute living things.

If it is not easy to get the water, mud and dead grass, fairly good results are obtained by putting some ordinary hay in water of any kind.

With fine forceps take a leaf or piece of stem of the dead grass and put it on a slide. Move it around and press it down so that a good drop of liquid and debris will be on the slide. Remove the grass and cover the liquid with a 0.15 mm. cover-glass. This should be studied fresh with a 4 mm. (40x) objective, 10x ocular, and transmitted light. Then put in place the dark-ground illuminator, center it and add some homogeneous liquid to the top of the condenser and run it up till the liquid is in contact with the under side of the slide.

Put a drop of homogeneous liquid on the cover-glass and use a homogeneous immersion objective in which the aperture has been cut down to 0.85 N.A. or less.

(2) Saliva. Put a drop of saliva on a slide and cover it with a 0.15 mm. cover-glass. Examine as in (1).

Note the pedetic or Brownian movement of the granules in the rounded salivary corpuscles, the minute granules in the broad oval epithelium, etc.

(3) *Fresh blood.* — For preparing and studying this, follow the directions given in § 211.

§ 351. Difference of appearance due to difference of focus. — If one takes a geometrical pattern like that shown in fig. 141 and looks at it in the ordinary way, the appearance is that of white spots on a dark field. If now the head is held closer and closer to the picture, an inversion will take place and the appearance is of dark spots in a white field. This illustrates how difficult it is to determine the real appearance under the microscope of objects having geometrical patterns, especially if there are several of them superimposed, as with the wire gauze experiment (§ 355). The image is often just as satisfactory in one focus as in another, although the appearance changes very markedly in the two positions.

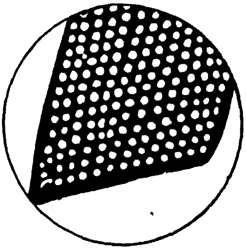


FIG. 141. GEOMETRICAL PATTERN TO SHOW DIFFERENCE OF APPEARANCE DEPENDING ON THE FOCUS.

(From Sir A. R. Wright's Microscopy).

§ 352. Comparing two microscopic fields side by side. — It is so difficult to carry in the mind the exact appearance of any structure or complex pattern, that many efforts have been made to have the microscopic images side by side so that they can be looked at at the same time. This has been accomplished by using two microscopes and projecting two fields side by side, as can be done by having two microscopes like the one shown in fig. 182.

Another method is by means of a comparison ocular (fig. 142). Then two objects under two microscopes have the images side by side in the ocular, half the field being taken up by one object and half by the other; then the eye can compare two structures side by side.

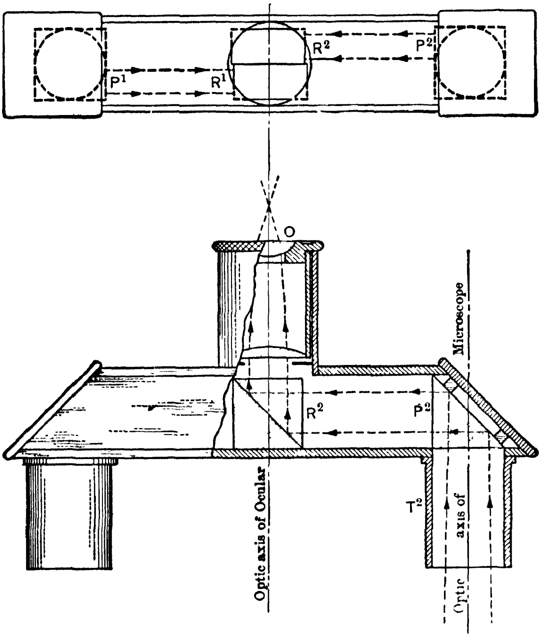


FIG. 142. COMPARISON OCULAR FOR PLACING HALF THE FIELDS OF TWO MICROSCOPES SIDE BY SIDE. (R^1 R^2).
(Bausch & Lomb Optical Co., from Chamot).

T^1 To fit into the tube of the left microscope.
T^2 To fit into the tube of the right microscope.
P Prism to reflect the beam from the right microscope to the prism R^2, whence it is reflected up through the ocular (O) into the right half of the field shown above in the face view.
P^1 R^1 The prism and left half of the field shown in face view in the diagram at the top.

§ **353. Muscæ volitantes.** — These specks or filaments in the eyes due to minute shreds or opacities of the vitreous humor, sometimes appear as part of the object as they are projected into the field of vision. They may be seen by looking into the well-lighted microscope. They may also be seen by looking at brightly illuminated snow or other white surface. By studying them carefully it will be seen that they are somewhat movable and float across the field of vision, and thus do not remain in one position as do the objects under observation. Furthermore, one may, by taking a little pains, familiarize himself with the special forms in his own eyes so that the more conspicuous at least may be instantly recognized.

§ **354. Miscellaneous observations.** — In addition to the above experiments it is very strongly recommended that the student follow the advice of Beale, p. 248, and examine first with a low power then with a higher power; mounted dry, then in water; lighted with reflected light, then with transmitted light, the following: potato, wheat, rice, and corn starch (easily obtained by scraping the potato and the grains mentioned); bread crumbs; portions of feather (portions of feather accidentally present in histological preparations have been mistaken for lymphatic vessels — Beale, 288); fibers of cotton, linen, and silk (textile fibers accidentally present have been considered nerve fibers, etc.); the scales of butterflies and moths, especially the common clothes moths; the dust swept from carpeted and wood floors; tea leaves and coffee grounds; dust found in living rooms and in places not frequently dusted (in the last will be found a regular museum of objects).

§ **355. Wire gauze experiment.** — For a very striking illustration of the need of care in interpretation with naked eye observation, take two pieces of wire gauze such as is used for milk strainers, or some slightly coarser. Place these over each other and look through them toward the light. Where there is but a single layer the weave is evident, but where the two pieces overlap the appearance is very puzzling, and changes constantly as one piece is rotated, bringing the threads and meshes at an angle. One could hardly believe that the structure is so simple when looking through two layers of the gauze.

If it is necessary then to see all sides of an ordinary gross object, to observe it in various positions and with varying illumination and under various conditions of temperature, moisture, and in single as well as multiple layers to obtain a fairly accurate and satisfactory knowledge of it, so much the more is it necessary to be satisfied with the interpretation of appearances under the microscope only after applying every means of investigation at command. Even then only such details of the image will be noted and understood as the brain behind the eye has been trained to appreciate.

§ 355a. **Experiment with wire gauze.** — För this very striking, naked-eye experiment with the wire gauze the author is indebted to a suggestion from Dr. Chamot.

§ 356. **Inversion of the microscopic image.** — As all the images produced by the modern compound microscope are inverted unless they are erected by a special arrangement of prisms, one must learn to interpret the appearances in an inverted image with the same certainty as in erect images seen by the naked eye or through the simple microscope. It may be remarked in passing that with the compound microscope the image is actually erect on the retina of the eye (figs. 2, 18).

With the compound microscope it soon becomes as easy to move the slide in the right direction to see a desired part as it is to make the proper motions when examining an object with the naked eye, although the motions are directly opposite in the two cases. Indeed, so natural does it become for the worker with the compound microscope to make the proper motions for the object giving the inverted image, that if he uses a compound microscope with an erecting prism he almost invariably moves the preparation in the wrong direction. With the simple microscope, however, it seems like naked-eye observation and there is never any difficulty.

This goes to show that by experience it is as easy to interpret inverted as erect images. This is further illustrated by the printer who learns to read type without difficulty, although it is a great puzzle to one who has learned to read the appearances only after the type has been printed on paper.

§ 357. **Physical analysis by the dark-field, the polarizing and the ultra-violet microscope.** — If one looks at objects with the bright-field microscope only, there may seem to be a complete revelation of the structure and form. But how inadequate that revelation is will become apparent if one or more of the special microscopes are used. For the application of these special microscopes, see Chapters III, IV and VI. Do not fail to get the additional information if the instruments are available.

§ 358. **Summary for proper interpretation.** — To summarize this chapter and leave with the beginning student the result of the experience of many eminent workers:

(1) Get all the information possible with the unaided eye. See the whole object and all sides of it, so far as possible.

(2) Examine the preparation with a simple microscope in the same thorough way for additional detail.

(3) Use a low power of the compound microscope.

(4) Use a higher power.

(5) Make sure that the mirror is in the best position to give the most favorable light. Vary the aperture by opening and closing the iris diaphragm to find the aperture which gives the clearest image in each case.

(6) Shade the top of the stage of the microscope to cut off the light from above and thus avoid confusion from that source.

(7) Use the highest power available and applicable. In this way one sees the object as a whole and progressively more and more details.

(8) If one has the apparatus, it is a good plan to examine specimens with a binocular microscope to gain the best notion possible of the relative position of parts of the specimen.

(9) Use the dark-ground illuminator (§ 349), the spectroscope, the polariscope, and the ultra-violet microscope (§§ 312–318, 357).

(10) Try staining the preparations to be studied in various ways to bring out the structural details; remember also the advantage of a color picture over a pure refraction image (§ 152) and especially of a combined color and refraction image. Keep in mind also that the microscopic image cannot be expected to reveal structural details that are not in some way clearly differentiated in the specimen.

(11) If artificial light must be used, employ a screen of daylight glass (§ 76) between the source of illumination and the microscope; then one can obtain true color effects.

(12) The composite picture derived from all available means of observation is much more likely to be correct than that obtained by only one or two means of observation.

(13) According to Wright, p. 46, it is far more difficult to prepare and illuminate a specimen properly than to get a good image of it after it is thus prepared and lighted.

COLLATERAL READING FOR CHAPTER VII

For general discussions: Carpenter-Dallinger; A. E. Wright, Principles of Microscopy, Ch. V; Beale; Spitta, Microscope, Ch. XVIII; Chamot, Chemical Microscopy.

For pedesis, see Jevons in Quart. Jour. Science, n.s., Vol. VIII (1878), p. 167; Rutherford, Science, N. S. Vol. XXX, 1909, pp. 289–302. For the original account of this see Robert Brown, "Botanical appendix to Captain King's voyage to Australia," Vol. II, p. 534 (1826).

For overcoming pedesis for photography see Gage, The use of a solution of gelatin to obviate pedesis in photographing milk globules and other minute objects in water, Transactions Amer. Micr. Soc., Vol. XXIV, 1902, p. 21.

For figures (photo-micrographs, etc.) of the various forms of starch, see Bulletin No. 13 of the Chemical Division of the U. S. Department of Agriculture. For hair and wool, see Bulletin of the National Association of Wool Growers, 1875, p. 470; Proc. Amer. Micro. Soc., 1884, pp. 65–68; Herzfeld, translated by Salter, The technical testing of yarns and textile fabrics, London, 1898.

HAUSMAN, L. A. — A micrological investigation of hair structure of the monotremata. Amer. Jour. Anat., Vol. 27, 1920, pp. 463–488. Many figs.

For different appearances due to the illuminator, see Nelson, in Jour. Roy. Micr. Soc., 1891, pp. 90–105; and for the illusory appearances due to diffraction phenomena, see Carpenter-Dallinger, p. 434; Mercer, Trans. Amer. Micr. Soc., V. 18 p. 321–396; also, A. E. Wright's Principles of Microscopy, especially the first five chapters; and chapter IX and the appendix. Conrad Beck. The Theory of the Microscope. Cantor Lectures before the Royal Society of Arts, Nov. Dec., 1907. 59 pages, London, 1908. See also collateral reading in previous chapters.

CHAPTER VIII

MAGNIFICATION AND MICROMETRY

§§ 359–398; FIGURES 143–166

WHY A MAGNIFIED IMAGE IS NECESSARY

§ **359.** The fundamental reason for using a microscope lies in the structure of the eye and its possibilities of adjustment for objects at different distances.

The sensory receptors or neuro-epithelium (rods and cones) of the eye stand in general with their long axes with the parallel rays of light entering the eye, hence the image of any external object falls on the ends of the sensory receptors. Now it is believed that if any image falls wholly upon one of the receptors, it will appear as a point; and if the image of two objects close together were to fall on one receptor, the two objects would appear as one.

§ **360.** Robert Hooke (1674), in dealing with the power of the human eye to distinguish double stars and to see two points or two details of an object as two, concluded that the two stars or the two points of any object must be at least far enough apart to make the visual angle one minute. A few people can distinguish double stars with a visual angle less than one minute, but for many people the visual angle must be greater. If the visual angle is too small, then the two stars or two points appear to fuse and form one. The visual angle of one minute then does not represent the limit of visibility, but the limit of resolution, that is, seeing two objects as two separate things.

Now as the visual angle under which any given object is seen depends upon its distance from the eye, and the power of accommodation for distance in the eye is limited, if very small objects are to be seen, or the parts of larger objects are to be distinguished as separate details, there must be some means of enabling the eye to get very close to the object.

The microscope serves to increase the visual angle under which an object is seen, thus virtually making it possible to get the eye very close to the object and still retain the sharpness of the retinal image. Or to put it in another way, the microscope helps the eye to produce a larger retinal image, and makes the details large enough to fall on more than one of the retinal elements, thus making resolution possible.

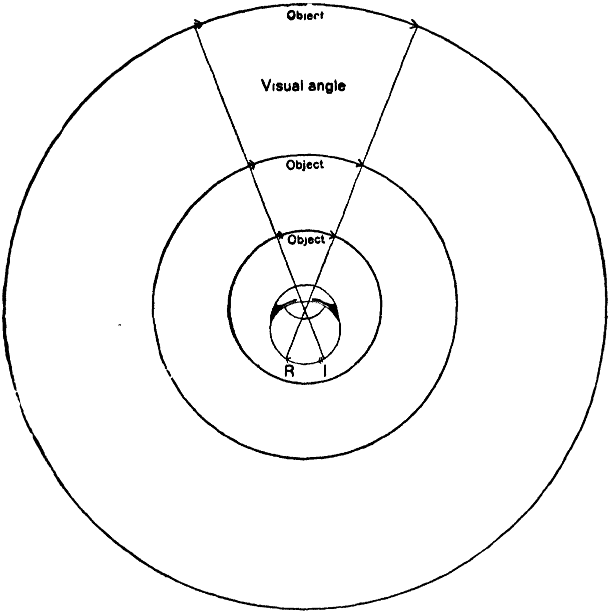


FIG. 143. CONSTANT RETINAL IMAGE (*R I*) AND CONSTANT VISUAL ANGLE WITH VARYING SIZE OF OBJECT AT DIFFERENT DISTANCES.

R I Retinal image. To keep this of constant size the visual angle must remain constant.

Object The object varying in size directly as the radius to keep the visual angle and the retinal image constant.

The radii in this figure are in the proportion of 1, 2, 4.

The sensory receptors of the retina — the rods and cones — are quite close together and over the greater part of the retina are commingled, there being more rods than cones. In the region of greatest visual acuity (fovea centralis of macula lutea), only cones are present. In general the rods are 2μ and the cones 6μ in diameter. In the fovea, however, the cones are slender, being only about 2μ to 3μ in diameter. These sizes give a clue to the size the retinal image must have in order that there be resolution, that is, that two points appear as two or two lines appear as two.

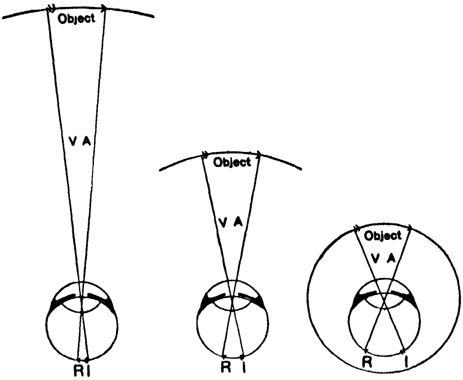


FIG. 144. CONSTANT SIZE OF OBJECT, THE VISUAL ANGLE AND THE RETINAL IMAGE VARYING WITH THE DISTANCE.

R I The retinal image varying inversely as the distance of the object.

V A The visual angle varying with the distance of the object from the eye.

Object The object of constant size but varying distance from the eye. The distance of the object is in the ratio of 1, 2, 4. The entire circle is shown at the right, but only a small arc in the other figures.

If we assume that Hooke was correct in the assumption that for two points to appear as two a visual angle of 1 minute is necessary, the diameter in millimeters or inches of the object, or the separation of the two points to render them visible as two, is easily determined as follows.

The nodal point or optic center of the eye is considered to be at the center of a circle (fig. 143), and the object at the circumference. No matter how great or how small the visual distance, the object must subtend one minute of the arc of the circle at whose circumference it is situated, in order that its two extremities shall appear separate. And so with any two details; they must be far enough apart to make the visual angle one minute.

To determine the actual length in millimeters required to subtend one minute of arc in any case, it is necessary to remember only that the entire circumference is 6.2832 times its radius ($2\pi r$), and that this circumference is divided into 360° or 21,600 minutes.

If, now, the radius of the circle, or the distance of the eye from the object, is 1 meter, the circumference of the circle will be 6.2832 meters or 6283.2 millimeters. As there are 21,600 minutes in the entire circumference, the actual length of one minute with a circle having a radius of one meter is 6283.2 mm. divided by 21,600 equals 0.29088 mm. That is, the eye at one meter distance requires two points or two lines to be separated a distance of 0.29088 mm. in order that they may be seen as two and not appear to be fused together.

It is assumed by workers with the microscope that the distance of most distinct vision for adults when looking at objects for details of structure is 254 mm. or 10 inches. This is the standard distance selected for the determination of magnifying power in microscopy also.

The question now is, how large a retinal image will be formed by an object giving a visual angle of 1 minute at the standard distance of 254 mm.

First must be found the actual size of the object to give a visual angle of 1 minute at 254 mm. distance. It is known from the above calculation that for one meter or 1000 mm. the object must have a size of 0.29088 mm. Now for 254 mm. the length must be $\frac{254}{1000}$ of this number or 0.07388352 mm., that is, a little more than one-fourth the size at 1 meter.

Now to determine the size of the retinal image at 254 mm. image distance, the distance from the center or nodal point of the eye must be known as well as the image distance and the size of the object. The distance of the retinal image from the nodal point is assumed to be 15 mm. (Howell, p. 311); then the size of the retinal image will be: $0.07388352 : x :: 254 : 15 = 0.00436$ mm. or 4.36μ, and this size would make the image fall on at least two of the cones of the fovea, and therefore there would be resolution and any two points would appear as two and not as one.

§ **361. The magnification, amplification, or magnifying power** of a simple or compound microscope is the ratio between the apparent and real size of the object examined. The apparent size is obtained by measuring the virtual image (figs. 145–146). For determining magnification the object must be of known length and is designated a micrometer (§ 366). In practice a virtual image is measured by the aid of some form of camera lucida (figs. 149, 169), or by double vision (§ 363). As the length of the object is known, the magnification is easily determined by dividing the size of the image by the size of the object. For example, if the virtual image measures 40 mm. and the object magnified, 2 mm., the amplification is 40 ÷ 2 = 20, that is, the apparent size is twentyfold greater than the real size.

Magnification is expressed in diameters or times linear; that is, but one dimension is considered. In giving a scale at which a microscopical or histological drawing is made, the word "magnification" is frequently indicated by the sign of multiplication: thus, ×450 upon a drawing means that the figure or drawing has the width or length of every detail 450 times as great as the object.

§ 362. Magnification of real images. — In this case the magnification is the ratio between the size of the real image and the size of the object, and the size of the real image can be measured directly. By recalling the work on the function of an objective, it will be remembered that it forms a real image on the ground-glass placed on the top of the tube, and that this real image could be looked at

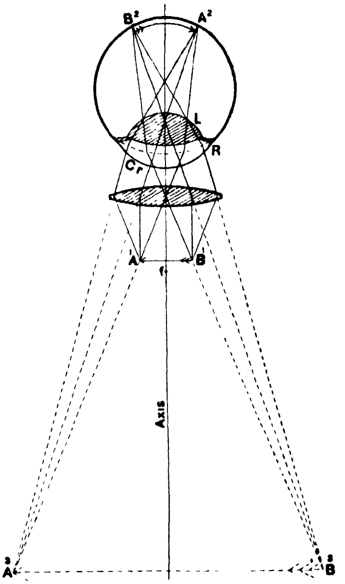


FIG. 145. SIMPLE MICROSCOPE WITH THE VIRTUAL IMAGE AT 250 MM. FROM THE EYE.

Axis The principal optic axis of the microscope and of the eye.

f The principal focus of the microscope.

$A^1 B^1$ The object just above the focus (*f*).

$B^2 A^2$ the retinal image; it is inverted.

$A^3 B^3$ The virtual image at 250 mm. from the eye; it is erect.

Cr Cornea of the eye.

R Single refracting surface of the schematic eye.

L The crystalline lens of the eye.

Axis The principal optic axis of the microscope and of the eye.

f f Principal focus of the objective, and of the ocular, *r im*, the real image formed by the objective just above the principal focus of the ocular.

cr The cornea of the eye.

rs The single refracting surface of the schematic eye.

l The crystalline lens of the eye.

r i The retinal image; it is erect.

The tube-length of the microscope (fig. 26) is 160 millimeters, and the image distance of the virtual image, 250 millimeters. For more complete explanation see fig. 18.

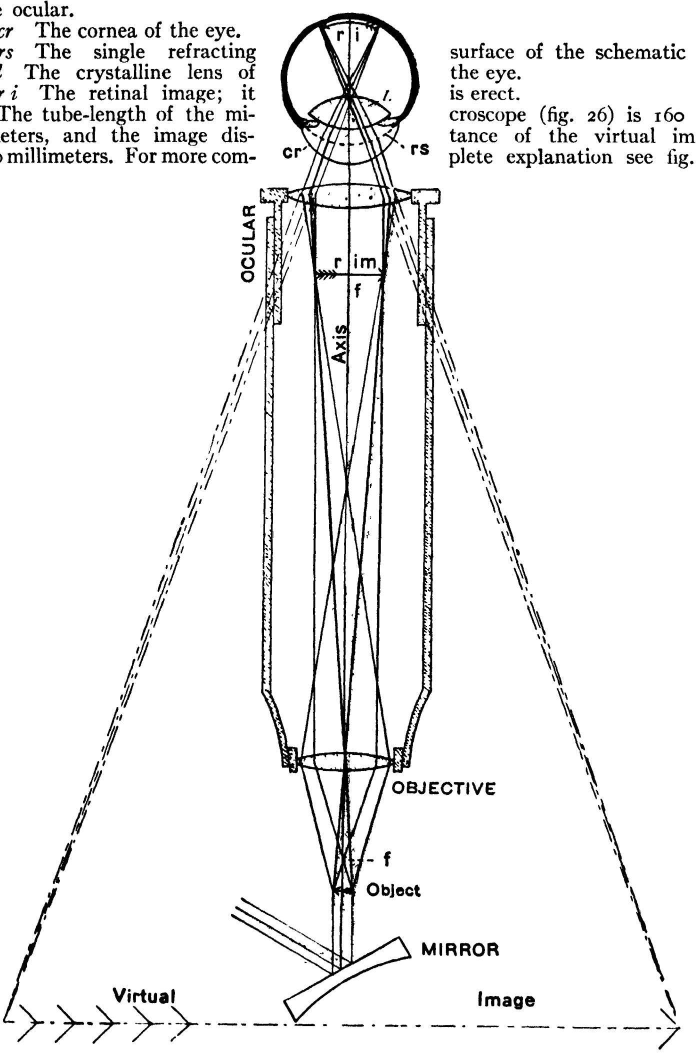


FIG. 146. COMPOUND MICROSCOPE SHOWING ALL THE IMAGES.

with the eye or measured as if it were an actual object. For example, suppose the object were three millimeters long and its image on the ground-glass measured 15 mm., then the magnification is $15 \div 3 = 5$, that is, the real image is 5 times as long as the object. The real images seen in photography are mostly smaller than the objects, but the magnification is designated in the same way by dividing the size of the real image measured on the ground-glass by the size of the object. For example, if the object is 400 millimeters long and its image on the ground-glass is 25 millimeters long, the ratio is $25 \div 400 = \frac{1}{16}$. That is, the image is $\frac{1}{16}$ as long as the object and is not magnified but reduced. In marking negatives, as with drawings, the sign of multiplication is put before the ratio, and in the example the designation is $\times \frac{1}{16}$. In photography and when using the magic lantern and the projection microscope, the images are real, and may be measured on the screen as if real pictures (fig. 147).

§ **363. The magnification of a simple microscope** is the ratio between the virtual image (figs. 6, 145, A^3B^3) and the object magnified (A^1B^1). To obtain the size of this virtual image, place the tripod magnifier near the edge of a support or block of such a height that the distance from the upper surface of the magnifier to the table is 250 millimeters.

As object, place a scale of some kind ruled in millimeters on the support under the magnifier. Put some white paper on the table at the base of the support and on the side facing the light.

Close one eye, and hold the head so that the other will be near the upper surface of the lens. Focus if necessary to make the image clear. Open the closed eye and the image of the rule will appear as if on the paper at the base of the support. Hold the head very still, and with dividers get the distance between any two lines of the image. This is the so-called method of double vision in which the microscope image is seen with one eye and the dividers with the other, the two images appearing to be fused in a single visual field.

§ **364. Measuring the spread of the dividers.** — This should be done on a steel scale divided to millimeters and $\frac{1}{5}$ mm.

As $\frac{1}{5}$ mm. cannot be seen plainly by the unaided eye, place one arm of the dividers at a centimeter line, and with the tripod magnifier count the number of spaces on the rule included between the points of the dividers. The magnifier simply makes it easy to count

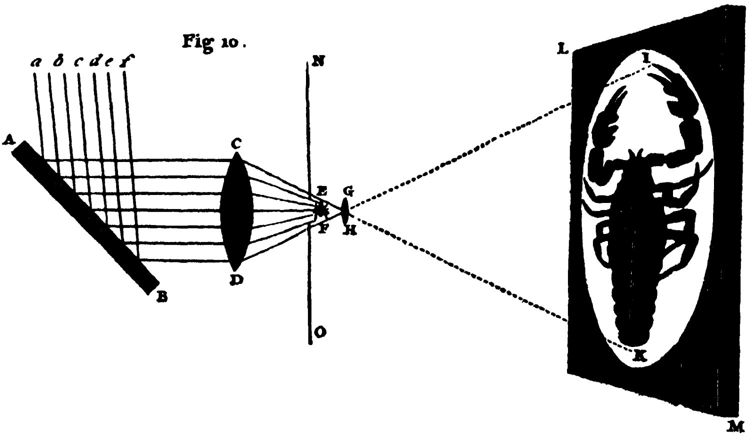


Fig. 147. Real Image Formed by a Projection Microscope.
(From the Essays of George Adams).

A B	Mirror reflecting the parallel rays of the sun upon the condenser (*C D.*)
a b c d e f	Parallel beams of light.
C D	The condenser.
N O	The stage of the projection apparatus.
E F	The object.
G H	The projection objective.
L M	The screen upon which the real image is shown.
I K	The real image of the object (*E F*).

the space on the rule included between the points of the dividers — it does not, of course, increase the number of spaces or change their value.

As the distance between the points of the dividers gives the size of the virtual image (fig. 145), and as the size of the object is known, the magnification is determined by dividing the size of the image by the size of the object. Thus, suppose the distance between the two lines at the limits of the image is measured by the dividers and found on the steel scale to be 15 millimeters, and the actual size of the space between the two lines of the object is 2 millimeters, then the magnification is 15 ÷ 2 = 7.5; that is, the image is 7.5 times as long or wide as the object. In this case the image is said to be magnified 7.5 diameters, or 7.5 times linear.

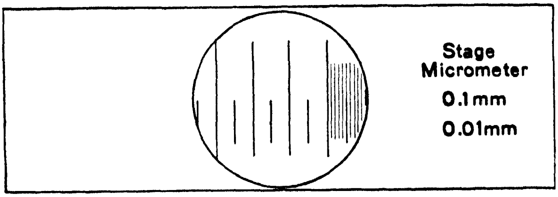


FIG. 148. STAGE MICROMETER RULED ON A COVER-GLASS.
The tenths millimeter (0.1 mm.) spaces are divided by short lines making the whole micrometer one with 0.1, 0.05, and 0.01 millimeters.

The magnification of any simple magnifier may be determined experimentally in the way described for the tripod magnifier; but this method is, of course, only possible when the observer has two good eyes. If he has but one eye, or his eyes are very unlike, then the magnification can be determined with one eye by using a camera lucida or the eikonometer (§§ 367, 390).

§ **365. The magnification of a compound microscope** is the ratio between the final or virtual image and the object magnified.

The determination of the magnification of a compound microscope may be made as with a simple microscope (§ 363), but this is fatiguing and unsatisfactory.

§ **366. Stage or object micrometer.** — For determining the magnification of a compound microscope and for the purposes of micrometry, it is necessary to have a finely divided scale or rule on glass or on metal. Such a finely divided scale is called a micrometer, and for ordinary work one mounted on a glass slide (1 × 3 in., 25 × 76 mm.) is most convenient.

The spaces between the lines should be 0.1 and 0.01 mm. (or if in inches, 0.01 and 0.001 in.). Micrometers are sometimes ruled on the slide, but more satisfactorily on a cover-glass of known thickness, preferably 0.15–0.18 mm. The covers should be perfectly clean before ruling, and afterwards simply dusted off with a camel's hair duster, and then mounted, lines downward over a shellac or other good cell (§ 525). If one rubs the lines the edges of the furrow made by the diamond are likely to be rounded and the sharpness of the micrometer is lost. If the lines are on the slide and uncovered one cannot use the micrometer with an oil immersion, as the oil obliterates the lines. Cleaning the slide makes the lines less sharp, as stated. If the lines are coarse, it is an advantage to fill them with plumbago or graphite. This may be done with some very fine plumbago on the end of a soft cork, or by using a soft lead pencil. Lines properly filled may be covered with balsam and a cover-glass as in ordinary balsam mounting (§ 533).

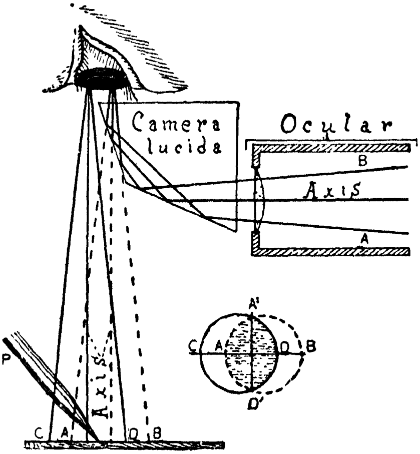


FIG. 149. WOLLASTON'S CAMERA LUCIDA.

§ 367. Determination of magnification. — This is most readily accomplished by the use of some form of camera lucida, that of Wollaston being most convenient, as it may be used for all powers, and the determination of the standard distance of 250 millimeters at which to measure the images is readily accomplished (fig. 149).

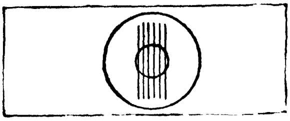


FIG. 150. STAGE MICROMETER WITH A RING ON THE LINES.

Employ the 16 mm. (10x) objective and a 5x ocular with a stage micrometer as object. For this power the 0.1 mm. spaces of the micrometer should be used as object. Focus sharply.

It is somewhat difficult to find the micrometer lines. To avoid this it is well to have a small ring enclosing some of the micrometer lines (fig. 150). The light must also be carefully regulated. If too much light is used, i.e., too large an aperture, the lines will be drowned in the light. In focusing with the high powers be very careful. Remember the micrometers are expensive and one cannot afford to break them. As suggested above, focus on the edge of the cement ring enclosing the lines; then, in focusing down to find the lines, move the preparation very slightly, back and forth. This will bring the lines into the field and the shadow made by them will indicate their presence, and one can then focus until they are sharp.

After the lines are sharply focused, and the slide clamped in position, make the tube of the microscope horizontal by bending the flexible pillar, being careful not to bring any strain upon the fine adjustment (fig. 26).

Put a Wollaston camera lucida (fig. 149) in position, and turn the ocular around if necessary so that the broad flat surface may face directly upward, as shown in the figure. Elevate the microscope by putting a block under the base, so that the perpendicular distance from the upper surface of the camera lucida to the table is 250 mm. (§ 370). Place some white paper on the worktable beneath the camera lucida.

Close one eye, and hold the head so that the other may be very close to the camera lucida. Look directly down. The image will appear to be on the table. It may be necessary to readjust the focus after the camera lucida is in position. If there is difficulty in seeing both dividers and image, consult § 408. Measure the image with dividers and obtain the power exactly as above (§ 364).

Thus: If two of the 0.1 mm. spaces are taken as object and the image is measured by the dividers, and the spread of the dividers is found on the steel rule to be 9.4 millimeters, the magnification (which is the ratio between size of image and object) is $9.4 \div 0.2 = 47$. That is, the magnification is 47 diameters, or 47 times linear.

Put the 10x ocular in place of the 5x, and then put the camera lucida in position. Measure the size of the image with dividers and a rule as before. The power will be considerably greater than when the low ocular was used. That is, the virtual image (fig. 146) seen with the high ocular is larger than the one seen with the low one.

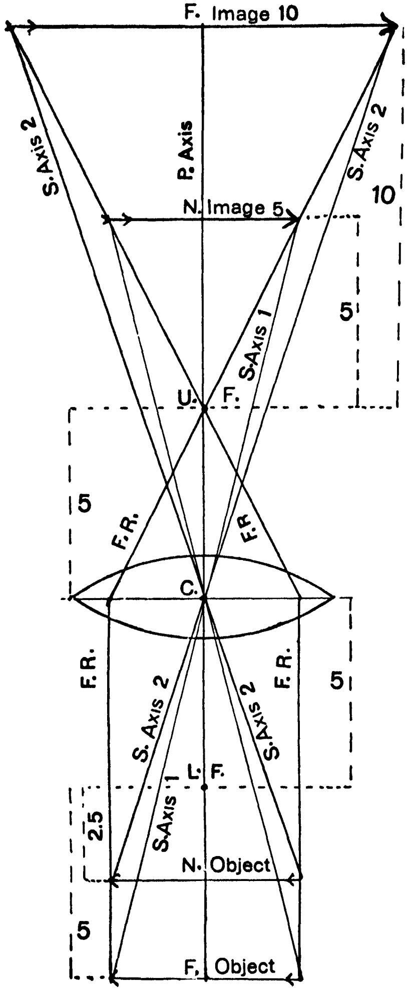


Fig. 151. Diagram to Show the Sizes of the Images When the Object Is at Different Distances from the Principal Focus.

The farther the object from the principal focus (*L. F.*) the nearer the image to the lens (*F. Object, N. Image*), and the nearer the object to the focus the farther will be the image from the lens (*N. Object, F. Image*). The sizes of the images will vary directly as their distances from the upper or conjugate focus (*U. F.*).

Lengthen the tube of the microscope 50–60 mm. by pulling out the draw-tube. Remove the camera lucida and focus; then replace the camera and obtain the magnification. It is greater than with the shorter tube. That is, the real image (fig. 151) is formed farther from the objective when the tube is lengthened, and the objective must be brought nearer the object. The law is: the magnification varies directly with the relative distance of the image and object from the center of the lens (fig. 152).

§ 368. Varying the magnification of a microscope. — There are five ways of varying the power of a compound microscope:

(1) By using a higher or lower objective.

(2) By using a higher or lower ocular.

(3) By lengthening or shortening the tube of the microscope.

(4) By increasing or diminishing the distance at which the virtual image is projected (fig. 153).

(5) By changing the relative position of the combinations in an adjustable objective (§§ 29, 149) or by the use of an amplifier (§ 369).

§ **369. Amplifier.** — In addition to the methods of varying the magnification given in § 368, the magnification is sometimes increased by the use of an amplifier, that is, a diverging lens or combination placed between the objective and ocular and serving to give the image-forming rays from the objective an increased divergence. An effective form of this accessory was made by Tolles, who made it as a small achromatic concavo-convex lens to be screwed into the lower end of the draw-tube (fig. 26) and thus but a short distance above the objective. The divergence given to the rays usually increases the size of the real image about twofold.

§ **370. Standard distance at which the virtual image is measured.** — For obtaining the magnification of both the simple and the compound microscope the directions were to measure the virtual image at a distance of 250 millimeters. That is, some standard distance must be chosen so that different workers can compare their results. The magnification could be found at almost any distance, and in getting the magnification of drawings the image distance is rarely ex-

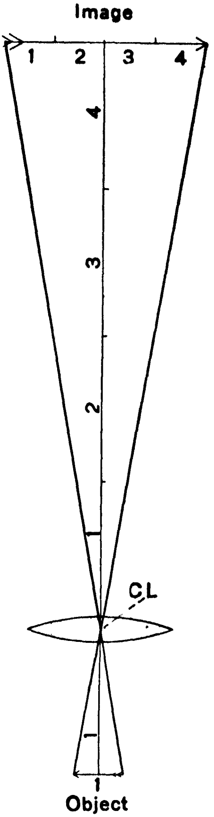


FIG. 152. TO SHOW THAT THE SIZE OF THE REAL IMAGE DEPENDS UPON ITS RELATIVE DISTANCE FROM THE CENTER OF THE OBJECTIVE.

Object 1 The object one unit of distance from the center of the lens (*CL*).

Image 1, 2, 3, 4 The image four units of distance from the lens and hence four times as long as the object.

actly 250 millimeters. Whenever the magnification of the microscope as a whole or of the objective or the ocular is mentioned, however, it is always understood that this magnification is at the standard distance of 250 mm. The necessity for the adoption of some common standard will be seen at a glance in fig. 153, where is represented graphically the fact that the size of the virtual image depends directly on the distance at which it is projected, and this size is directly proportional to the vertical distance from the apex of the triangle of which it forms a base. The distance of 250 millimeters has been chosen on the supposition that it is the distance of most distinct vision for normal adults when examining details.

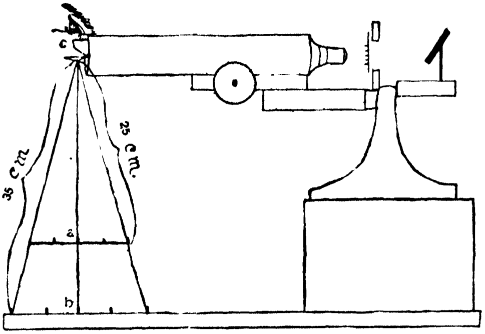


FIG. 153. DIAGRAM TO SHOW THAT THE SIZE OF THE VIRTUAL IMAGE DEPENDS UPON THE PROJECTION DISTANCE.

a Size of image at a projection distance of 25 cm.
b Image at 35 cm.
The sizes are directly as the projection distances.
C The camera lucida and under it a spectacle lens to aid the eye in focusing the pencil point; this is only needed by those with defective eyes.

In preparing drawings it is often of great convenience to make them at a distance less or greater than the standard. In that case the magnification must be determined for the image distance actually used.

§ 371. Magnification and relation of the object to the principal focus. — As shown by figures 154 and 155, independent of the equivalent focus of the simple microscope or the objective, the real image or the virtual image, as the case may be, will be larger the nearer the object is to the principal focal point.

In figure 156 it is shown also that if the object or the real image is in the plane of the principal focus, the rays emerging from the simple microscope or the ocular will be in parallel bundles, and when pro-

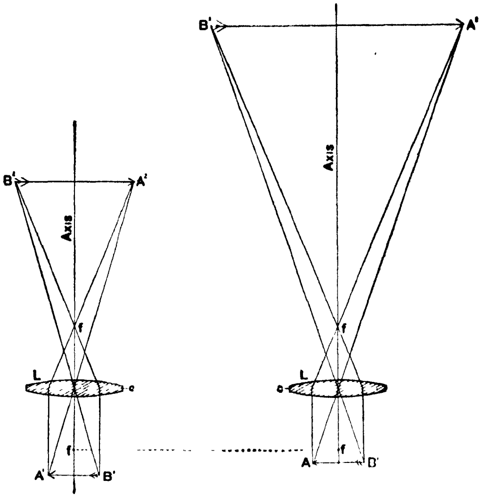


FIG. 154. DIAGRAMS TO SHOW THAT THE SIZE OF THE REAL IMAGE OF A LENS DEPENDS UPON THE DISTANCE OF THE OBJECT FROM THE PRINCIPAL FOCUS.

Axis The principal optic axis extended above and below. *A B*, *B A* The object and the inverted real image. *f*, *f* The principal focus above and below each lens. *Lc* The lens.

The object is the same size in the two cases, but the images differ, depending upon the distance of the object from the principal focus, being longer the nearer the object is to the focus.

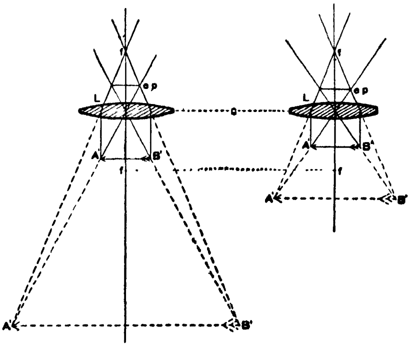


FIG. 155. DIAGRAMS TO SHOW THAT THE SIZE OF THE VIRTUAL IMAGE OF A LENS DEPENDS UPON THE DISTANCE OF THE OBJECT FROM THE PRINCIPAL FOCUS.

A B, *A B* The object and the virtual image. *f f* The principal focus. *L* The lens. *ep* The eyepoint. *c* The single, ideal refracting plane.

As with real images, the size of virtual image in a given lens depends upon the nearness of the object to the principal focus.

jected by the eye must also be in parallel bundles. It is further shown in such a case that the rays emanating from any point in the object or real image will not in that case form a virtual point focus at the standard distance of 250 mm., as shown in fig. 145, but will remain parallel. At that distance, then, the image on the retina would be a diffusion circle. In order that there be the appearance of a point focus the distance must be great enough so that the parallel rays from a point will be separated less than one minute (§§ 359-360).

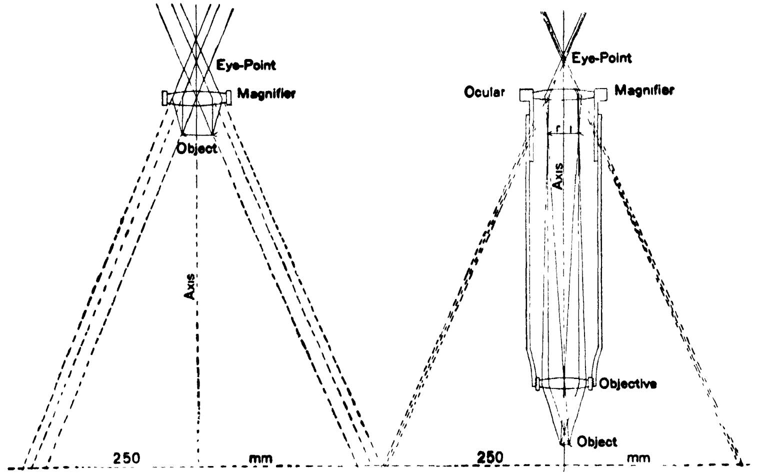


FIG. 156. DIAGRAMS OF SIMPLE AND COMPOUND MICROSCOPES WITH PARALLEL BEAMS EMERGING ABOVE AND PROJECTED BELOW.

Axis The principal optic axis.
Object The object.
Objective The objective of the compound microscope.
r i The real image formed by the objective.
Ocular-Magnifier The ocular and magnifier for the real image in the compound microscope, and for the object in the simple microscope.
Eyepoint The most favorable position for the eye of the observer.

Below, at 250 mm., the usual position of the projected image, no image is formed with parallel rays. These only seem to come from a point at a distance where their separation is less than one minute of arc (§ 359-360).

§ 372. Table of magnification and of the valuations of the ocular

micrometer. — The table should be filled out by each student. In using it for micrometry and drawing it is necessary to keep clearly in mind the exact conditions under which the determinations were made, and also the ways in which variations in magnification and the valuation of the ocular micrometer may be produced.

	OCULAR 5x		OCULAR 10x		OCULAR MICROMETER VALUATION	
OBJECTIVE	TUBE IN	TUBE OUT — MM.	TUBE IN	TUBE OUT — MM.	TUBE IN.	OUT—MM.
	×	×	×	×		
	×	×	×	×		
	×	×	×	×		
	×	×	×	×		
	×	×	×	×		
	×	×	×	×		
SIMPLE MICROSCOPE.	×					

OCULAR MICROMETER AND ITS VALUATION

§ **373.** This, as the name implies, is a micrometer to be used in connection with an ocular. It consists of rulings of fixed or of movable lines on a cover-glass.

This form cf micrometer is placed at the level where the real image is formed, i.e., at the level of the ocular diaphragm of all oculars. With positive oculars it would therefore be outside the ocular (figs. 22–23) and with negative or Huygenian oculars, between the lenses (figs. 24–25). The image of the object under the microscope appears to be directly upon or immediately under the ocular micrometer, and hence the number of spaces on the ocular micrometer required to measure the real image may be read off directly. This, however, is measuring the size of the real image, and the actual size of the object can be determined only by finding the ratio between the size of the real image and that of the object. In other words, it is necessary to get the valuation of the ocular micrometer in terms of a stage micrometer.

§ **374. Valuation of the ocular micrometer.** — This is the value of the divisions of the ocular micrometer for the purposes of micrometry, and is entirely relative, depending on the magnification of the real image formed by the objective; consequently it changes with every change in the magnification of the real image, and must be especially determined for every change modifying the real image of the microscope (§ 368).

It will be seen when the ocular micrometer valuation is found for different objectives, that the greater the magnification of the objective, the less will be the ocular micrometer valuation; and conversely, the less the magnification of the objective, the greater will be the ocular micrometer valuation.

§ **375. Obtaining the ocular micrometer valuation for an ocular micrometer with fixed lines.** — If the ocular micrometer is on a cover-glass, place it on the diaphragm of the 5x or 10x ocular after removing the eyelens. Screw the eyelens back in place, and put the ocular in the tube of the microscope. Put a 16 mm. (10x) objective in place. Use the stage micrometer as object. Light the field well and look into the microscope. The lines of the ocular micrometer should be very sharply defined. If they are not, raise or lower the eyelens to make them so; that is, focus as with the simple magnifier.

When the lines of the ocular micrometer are distinct, focus the microscope (§ 367) for the stage micrometer. The image of the stage micrometer appears to be directly under or upon the ocular micrometer.

Make the lines of the two micrometers parallel by rotating the ocular or changing the position of the stage micrometer or both if necessary, and then make any two lines of the stage micrometer coincide with any two on the ocular micrometer (fig. 157). To do this it may be necessary to pull out the draw-tube a greater or lesser distance. See how many spaces are included in each of the micrometers (figs. 157, 165).

Divide the value of the included space or spaces on the stage micrometer by the number of divisions on the ocular micrometer required to include them, and the quotient so obtained will give the valuation of the ocular micrometer. For example, suppose the millimeter is taken as the unit for the stage micrometer and this unit is divided into spaces of 0.1 and 0.01 millimeters. If with a given optical combination and tube-length it requires 10 spaces on the ocular micrometer to include the real image of 0.1 millimeter on the stage micrometer, obviously one space on the ocular micrometer includes only one-tenth as much, or 0.1 mm. ÷ 10 = 0.01 mm. That is, each space on the ocular micrometer includes 0.01 of a millimeter on the stage micrometer, or 0.01 millimeter of the length of any object under the microscope, the conditions remaining the same. Or, in other words, it requires 100 spaces on the ocular micrometer to include 1 millimeter on the stage micrometer; then, as before, 1 space of the ocular micrometer would have a valuation of 0.01 millimeter for the purposes of micrometry. The size of any minute object may be determined by multiplying this valuation of one space by the number of spaces required to include it. For example, suppose the fly's wing or some part of it covered 8 spaces on the ocular

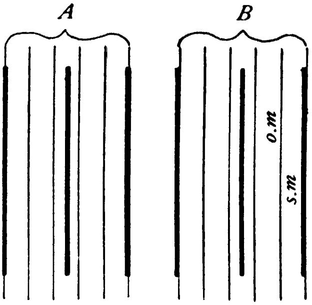


FIG. 157. THE IMAGES OF THE OCULAR AND OF THE STAGE MICROMETER, SHOWING HOW TO ARRANGE THE LINES.

o.m Ocular, *s.m* Stage micrometer lines.

A Lines of the ocular micrometer opposite the middle of the lines of the stage micrometer.

B Lines of the ocular micrometer at the right side of the lines of the stage micrometer (compare fig. 165.)

micrometer; it would be known that the real size of the part measured 0.01 mm. × 8 = 0.08 mm. or 80μ (§§ 380–382).

Proceed in exactly the same manner to get the ocular micrometer valuation when using any objective, whether it is of higher or lower power than the one in this section.

Any Huygenian ocular may be used as a micrometer ocular by placing the ocular micrometer at the level of the ocular diaphragm where the real image is formed. If there is a slit in the side of the ocular and the ocular micrometer is mounted properly, it may be introduced through the opening in the side. This was a common method with the older microscopes. When there is no side opening, the eyelens may be unscrewed and the ocular micrometer on a cover-glass laid upon the ocular diaphragm.

OCULAR MICROMETER WITH MOVABLE SCALE

§ 376. Ocular micrometer with movable scale. — The form here shown is a Huygenian ocular with a micrometer scale on the diaphragm of the ocular. The eyelens is adjustable up and down for focusing the scale, and a drum with 100 divisions is attached to the screw which moves the scale from side to side. Each interval on the drum represents 0.01th of a complete interval on the scale, thus enabling one to measure an object 0.01th the size of one requiring a whole scale-interval. This ocular micrometer combines the advantages of the ocular micrometer with fixed lines and the filar micrometer. To complete the measurement of an object not

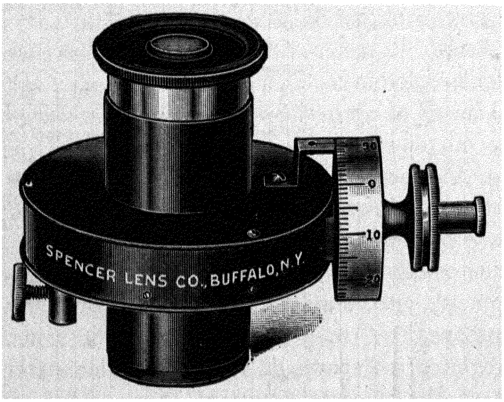


FIG. 158. OCULAR MICROMETER WITH MOVABLE SCALE AND RECORDING DRUM.

(From the Catalogue of the Spencer Lens Co.)

included exactly between any two lines of the scale, the drum need be revolved only partly around.

§ 377. **Valuation of the movable scale ocular micrometer (fig. 158).** — Use a 4 mm. (40x) objective and proceed exactly as for the micrometer with fixed lines, except that a partial stage micrometer space can be measured by rotating the drum until the ocular micrometer exactly coincides with the stage micrometer. Make sure that the lines of the two micrometers are correctly related, as shown in figs. 157 and 165. One can then count up the number of spaces on the ocular micrometer required to measure one or more spaces of the stage micrometer. To this is then added the $\frac{1}{100}$ spaces on the drum. For example, suppose that three 0.01 mm. spaces of the stage micrometer are taken as object, and that it requires seven complete spaces of the ocular micrometer and $\frac{50}{100}$ on the drum to include the three spaces on the stage micrometer; then each space on the ocular micrometer would be equal to 0.03 mm. divided by 7.50 = 0.004 mm. or 4μ. One of the spaces on the drum which represents one hundredth of an interval on the ocular micrometer would have a valuation under these conditions of 4μ divided by 100 = 0.04 microns. This gives a notion of the minuteness of the object which can be measured, and of the smallness of the error in measuring large objects, even if the observation erred in getting the object one or more of the drum divisions too large or too small.

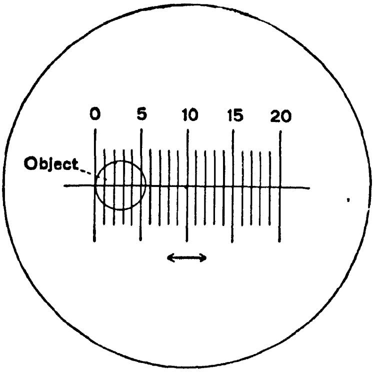


Fig. 159. Field of the Microscope Showing the Movable Scale of the Huygenian Micrometer Ocular (Fig. 158).

The arrow indicates that the scale may be moved in both directions.

0, 5, 10, 15, 20 These figures indicate the 20 spaces in groups of 5. Each space represents a total revolution of the screw (screw with $\frac{1}{4}$ mm. pitch). Each of the 100 divisions on the drum (fig. 91) represents then $\frac{1}{400}$ mm.

Object The circular object in the field measures 5 intervals on the ocular micrometer and 45 intervals on the drum, hence the entire diameter of the object is 5.45 intervals on the ocular micrometer.

For an actual measurement with this ocular micrometer, see § 387.

One would proceed exactly as above for getting the valuation with any other objective.

FILAR OCULAR MICROMETER

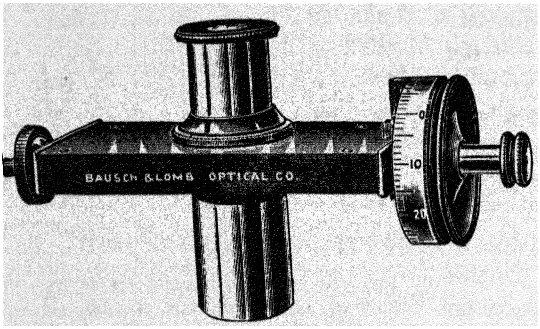


FIG. 160. FILAR MICROMETER OCULAR.
(From the 16th ed. of the Catalogue of the Bausch & Lomb Optical Co.).

This is a Ramsden ocular, and the recording drum is divided into 100 equal divisions, and as the pitch of the screw is 0.5 mm., each division on the drum represents an actual movement of 0.005 mm. of the movable line.

§ 378. This form of ocular micrometer usually consists of a Ramsden ocular with fixed cross lines and a movable line (fig. 161). For obtaining the valuation of this ocular micrometer proceed as follows: Employ a 4 mm. (40x) objective. Carefully focus the $\frac{1}{100}$ mm. lines. The lines of the ocular micrometer should also be sharp; if they are not, focus them by moving the ocular up or down in the sliding tube. Make the vertical lines of the ocular micrometer parallel with the lines of the stage micrometer (figs. 157, 165). Note the position of the graduated drum and the teeth of the recording comb, and then rotate the wheel until the movable line traverses one space on the stage micrometer. Each tooth of the recording comb indicates a total revolution of the wheel, and by noting the number of teeth required and the graduations on the wheel, the revolutions and part of a revolution required to measure the 0.01 mm. of the stage micrometer can be easily noted. Measure in like manner 4 or 5 spaces and get the average. Suppose this average is $1\frac{1}{4}$ revolutions or 125 graduations on the wheel, to measure the 0.01 mm. or 10μ (see §§ 380–382), then one of the graduaations on the wheel would measure 10μ divided by 125 = 0.08μ. In using this valuation for actual measurement, the tube of the microscope and the objective must be exactly as when obtaining the valuation (§§ 368–377).

The valuation of the filar micrometer can be obtained for any objective by proceeding exactly as above. (See § 388 for measurement.)

Micrometry is the determination of the size of objects by the aid of a microscope.

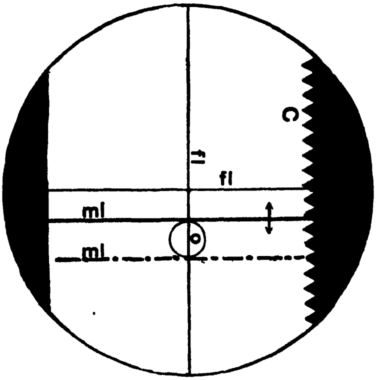


FIG. 161. FIELD OF THE MICROSCOPE SHOWING THE LINES AND THE RECORDING COMB OF THE FILAR MICROMETER (FIG. 160).

C The recording comb. Each tooth represents a complete revolution of the drum (fig. 160).

fl, *fl* The fixed cross lines.

ml, *ml* The movable line.

The arrow shows that the movable line can be moved in both directions.

O Object, the full movable line (*ml*) shows it at one edge of the object and the broken line shows it at the other edge of the object. The intervening teeth of the comb show that the screw was turned two whole revolutions and the recording drum showed 90 divisions, making two and nine tenths revolutions of the drum to carry the movable line from one edge of the object to the other.

MICROMETRY WITH THE SIMPLE MICROSCOPE

§ 379. With a simple microscope (1), the easiest and best way is to use dividers and then with the simple microscope determine when the points of the dividers exactly include the object. The spread of the dividers is then obtained as above (§§ 363–364). This amount will be the actual size of the object, as the microscope was used only in helping to see when the divider points exactly enclosed the object.

(2) One may put the object under the simple microscope and then, as in determining the power (§ 363), measure the image at the standard distance. If the size of the image so measured is divided by the magnification of the simple microscope, the quotient gives the actual size of the object. One might use the eikonometer also (§ 391).

Use a fly's wing or some other object of about that size and try to determine the width in the two ways described above. If all the work is done accurately, the results will agree.

MICROMETRY WITH THE COMPOUND MICROSCOPE

There are several ways of varying excellence for obtaining the size of objects with the compound microscope, the method with the ocular micrometer (§ 373) being most accurate.

§ **330. Unit of measure in micrometry.** — Most of the objects measured with the compound microscope, and many of those in physics and chemistry are smaller, often much smaller, than any of the originally named divisions of the meter. To express these very small dimensions in common or in decimal fractions of a meter or millimeter is not only cumbersome, but likely to give rise to errors; consequently workers in microscopy, in physics and in chemistry have sought to avoid the difficulties by selecting and naming as units such small divisions of the meter that the minute dimensions can be expressed as whole numbers.

The Micron unit (μ) has been generally adopted in microscopy, and is widely used for minute sizes in all branches of science. Harting recommended it for microscopy in 1859, but he named it micro-millimeter, or milli-millimeter, and gave as a symbol *mmm*. Since the definite meaning for *micro*, as one millionth of the unit before which it is placed, has been decided on by metrologists, micro-millimeter should mean one millionth of a millimeter, not one thousandth. Harting's Milli-millimeter is correct, but awkward. Occasionally one meets the symbol μμ for millimicron (mμ). μμ should stand for the millionth, not for the thousandth, of a micron.

Up to the present three such special units have been designated and have received the sanction and use of the highest authorities. They are:

§ **380a. 1. The Micron (symbol μ).** This is the one millionth of a meter (0.000001, m.); one thousandth of a millimeter (0.001 mm.); one thousand millimicrons (1000 mμ); ten thousand Angström units (10,000 Ä).

§ **381. 2. The Millimicron (mμ).** This is the one billionth of a meter (0.000 000 001 m.); the one thousandth of a micron (0.001 μ); ten Angström units (10 Ä.).

§ **382. 3. The Angström Unit (Ä.)** or Tenthmeter (10^{-10} m.). It is the one ten billionth of a meter (0.000 000 000 1 m); the ten thousandth of a micron (0.000 1 μ); the one tenth of a millimicron (0.1 mμ).

See Jour. Roy. Micr. Jour. Soc., 1888, p. 502. Nature, Vol. XXXVII, p. 388; Blt. Bureau Standards, Vol. VIII, p. 540.

§ **383. Micrometry by the use of a stage micrometer on which to mount the object.** — In this method the object is mounted on a micrometer and then put under the microscope, and the number of spaces covered by the object is read off directly. It is exactly like putting any large object on a rule and seeing how many spaces of the rule it covers. The defect in the method is that it is impossible to arrange objects properly on the micrometer. Unless the objects are circular in outline they are likely to be oblique in position, and in every case the end or edges of the object may be in the middle of a space instead of against one of the lines; consequently the size must be estimated or guessed at rather than really measured.

§ **384. Micrometry by dividing the size of the image by the magnification of the microscope.** — For example, employ the 4 mm. (40x) objective, and 5x or 10x ocular. For measurement use a preparation of the blood corpuscles of the frog, necturus, or other animal with large oval corpuscles. Obtain the size of the image of the long and short axes of three corpuscles with the camera lucida and dividers, exactly as in obtaining the magnification of the microscope (§ 367). Divide the size of the image in each case by the magnification, and the result gives the actual size of the blood corpuscles. Thus, suppose the image of the long axis of the corpuscle is 18 mm. and the magnification of the microscope 400 diameters (§ 361), then the actual length of this long axis of the corpuscle is 18 mm. ÷ 400 = 0.045 mm. or 45μ (§ 364).

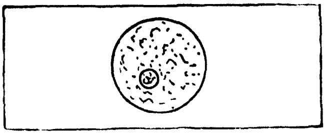


FIG. 162. BLOOD PREPARATION WITH A RING AROUND A GROUP OF CORPUSCLES.

As the same three blood corpuscles are to be measured in three ways, it is an advantage to put a delicate ring around a group of three or more corpuscles, and make a sketch of the whole enclosed group, marking on the sketch the corpuscles measured (fig. 162). The different corpuscles vary considerably in size, so that accurate comparison of different methods of measurement can be made only when the same corpuscles are measured in each of the ways.

§ **385. Micrometry by the use of a stage micrometer and a camera lucida.** — Employ the same object, objective and ocular as before. Put the camera lucida in position, and with a lead pencil make dots on the paper at the limits of the image of the blood corpuscles. Measure the same three that were measured in § 384.

Remove the object, place the stage micrometer under the microscope, focus well, and draw the lines of the stage micrometer so as to include the dots representing the limits of the part of the image to be measured. As the value of the spaces on the stage micrometer is known, the size of the object is determined by the number of spaces of the micrometer required to include it.

This simply enables one to put the image of a fine rule on the image of a microscopic object. It is theoretically an excellent method, and nearly the same as measuring the spread of the dividers with a simple microscope (§ 364).

§ 386. Micrometry with the ocular micrometer with fixed lines. — Use the 4 mm. (40x) objective, and the ocular with the ocular micrometer. For object use the same corpuscles as in §§ 384–385. Make sure that all the conditions are exactly as when the valuation was determined; then put the preparation under the microscope and find the same three red corpuscles that were measured in the other ways (§ 384).

Count the divisions on the ocular micrometer required to enclose or measure the long and the short axis of each of the corpuscles, multiply the number of spaces in both cases by the valuation of the ocular micrometer, and the results will represent the actual length of the axes of the corpuscles in each case.

The same corpuscle is, of course, of the same actual size, when measured in each of the three ways, so that if the methods are correct and the work carefully enough done, the same results should be obtained by each method.

§ 387. Micrometry with the movable scale ocular micrometer. — Use the same preparation and objective as before. Arrange the micrometer ocular so that the long axis of the corpuscle will coincide with the cross line in the micrometer scale (figs. 158–159). Get one end of the corpuscle exactly level with one division of the micrometer scale. Note the position of the drum, and then rotate it until the other end of the corpuscle is exactly against the nearest line of the micrometer. Count up the entire intervals required and the partial interval on the drum. Suppose it requires 5 entire and 0.60 intervals (see explanation of fig. 159); then the whole corpuscle must be 5.60 intervals multiplied by 4μ (§ 377), the value of one interval; $5.6 \times 4 = 22.4\mu$.

§ **388. Micrometry with the filar micrometer.** — Use the same preparation and objective as before, but use a filar micrometer. Note how many graduations on the recording comb and drum (fig. 160) are required to measure each dimension of the corpuscle, and multiply by the valuation as in the other cases.

The advantage of the filar micrometer is that the evaluation of one graduation is so small that even the smallest object to be measured would require several graduations to measure it. In ocular micrometers with fixed lines, small objects like bacteria might not fill even one space; therefore estimations, not measurements, must be made. For large objects, like most of the tissue elements, the ocular micrometers with fixed lines answer very well, for the part which must be estimated is relatively small and the chance of error is correspondingly small (§ 389).

§ **389.** There are three ways of using the ocular micrometer, or of arriving at the size of the objects measured with it:

(1) By finding the value of a division of the ocular micrometer for each optical combination and tube-length used, and employing this valuation as a multiplier. This is the method given in the text, and the one most frequently employed. Thus, suppose with a given optical combination and tube-length it required five divisions on the ocular micrometer to include the image of 0.2 millimeter of the stage micrometer, then obviously one space on the ocular micrometer would include $\frac{1}{5}$ or 0.2 or 0.04 mm.; the size of any unknown object under the microscope would be obtained by multiplying the number of the divisions on the ocular micrometer required to include its image by the value of one space, or in this case 0.04 mm. Suppose some object, as the fly's wing, required 15 spaces of the ocular micrometer to include some part of it, then the actual size of this part of the wing would be $15 \times 0.04 = 0.6$ mm.

(2) By finding the number of divisions on the ocular micrometer required to include the image of an entire millimeter of the stage micrometer, and using this number as a divisor. This number is also sometimes called the ocular micrometer ratio. Taking the same case as in (1), suppose five divisions of the ocular micrometer are required to include the image of 0.2 mm., on the stage micrometer,

then evidently it would require 5 ÷ 0.2 = 25 divisions on the ocular micrometer to include a whole millimeter on the stage micrometer, and the number of divisions of the ocular micrometer required to measure an object divided by 25 would give the actual size of the object in millimeters or in a fraction of a millimeter. Thus, suppose it required 15 divisions of the ocular micrometer to include the image of some part of the fly's wing, the actual size of the part included would be 15 ÷ 25 = $\frac{3}{5}$ or 0.6 mm. This method is really exactly like the one in (1), for dividing by 25 is the same as multiplying by $\frac{1}{25}$ or 0.04.

(3) By having the ocular micrometer ruled in millimeters and divisions of a millimeter, and then getting the size of the real image in millimeters. In employing this method a stage micrometer is used as object and the size of the image of one or more divisions is measured by the ocular micrometer, thus: Suppose the stage micrometer is ruled 0.1 and 0.01 mm. and the ocular micrometer is ruled in millimeters and 0.1 mm. Taking 0.2 mm. on the stage micrometer as object, as in the other cases, suppose it requires 10 of the 0.1 mm. spaces of 1 mm. to measure the real image, then the real image must be magnified 1.0 ÷ 0.2 = 5 diameters, that is, the real image is five times as great in length as the object, and the size of an object may be determined by putting it under the microscope and getting the size of the real image in millimeters with the ocular micrometer and dividing it by the magnification of the real image, which in this case is 5 diameters.

Use the fly's wing as object, as in the other cases, and measure the image of the same part. Suppose that it required 30 of the 0.1 mm. divisions = 3 mm. to include the image of the part measured, then evidently the actual size of the part measured is 3 mm. ÷ 5 = $\frac{3}{5}$ mm., or 0.6 mm., the same result as in the other cases. See also § 390 on the eikonometer.

In comparing these methods it will be seen that in the first two the ocular micrometer may be simply ruled with equidistant lines without regard to the absolute size in millimeters or inches of the spaces. In the last method the ocular micrometer must have its spaces some known division of a millimeter or inch. In the first two methods only one standard of measure is required, viz., the stage micrometer; in the last method two standards must be used, viz., a stage micrometer and an ocular micrometer.

§ 390. Eikonometer for magnification and micrometry. — The eikonometer is something like an eye. It has a converging lens serving in place of the crystalline lens to focus the rays from the eyepiece of the compound microscope, or from the simple microscope upon a micrometer scale, the scale taking the place of the retina in the eye (figs. 145–146). This scale is ruled in 0.1 mm. Above the scale is a Ramsden ocular of 25 mm. equivalent focus, giving a magnification of 10. The eikonometer scale, therefore, is a millimeter scale when seen at the distance of 250 mm. in the visual field of the normal human eye, and it enables one to put a millimeter scale on the image of any object studied.

To use it for magnification a stage micrometer is put under the microscope and carefully focused. Then the eikonometer is put in place over the ocular. The microscopic image of the stage micrometer and the scale of the eikonometer will then appear in the same field as with the ordinary ocular micrometer (§ 375). The two sets of lines should be made parallel (§§ 374–376). See how many divisions of the eikonometer millimeter scale are required to measure one or more of the divisions of the image of the stage micrometer. Suppose it requires 6 intervals or millimeters of the eikonometer scale to measure the image of 0.03 mm. on the stage micrometer. The size of the object is then 0.03 mm., and of its image 6 mm. The magnification is therefore (§ 361) 6 ÷ 0.03 = 200.

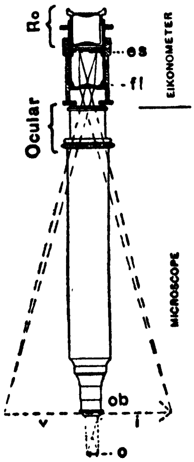


FIG. 163. WRIGHT'S EIKONOMETER.

(From Sir A. E. Wright's Principles of Microscopy).

o Object.
v i Virtual image.
ob Objective.

Microscope Ocular, the objective, tube and ocular of the microscope.

Eikonometer The Ramsden ocular (*Ro*) magnifying 10 diameters, and field lens (*fl*) above the ocular of the microscope.

es The real image formed at the diaphragm of the eikonometer.

For determining the magnification of a simple microscope the eikonometer is placed over the simple microscope as it was over the ocular above. With this instrument, as with the camera lucida, only one eye is used (figs. 149, 169).

§ **391. Micrometry with the eikonometer.** — In the first place the magnification of the microscope must be determined as described in the preceding section; and one must keep in mind the factors which will vary the magnification (§ 368). The object to be measured is put under the microscope and focused and the eikonometer put in position. The virtual image is then measured in millimeters by the scale of the instrument. The size of this virtual image is then divided by the magnification and the result will be the actual size of the object as in § 384.

For example, suppose the long axis of a necturus' red blood corpuscle measures 9 mm. on the eikonometer scale. If the magnification of the microscope is 200, as found above, then the actual length of the corpuscle is 9 mm. ÷ 200 = 0.045 mm., or 45μ.

§ **392. Micrometry by the aid of the condenser image of a scale.** — Probably every one is all too familiar with the cross bars of the window in the field of the microscope. This is, as well known, a real image of the window produced by the condenser at the level of the object. The possibility of projecting a real image at the level of the object is taken advantage of for purposes of micrometry as follows: A lantern slide is made of net lines (fig. 164) or of any parallel, equidistant lines. The lantern slide is then set up exactly 10 cm. or some other exact distance in front of the microscope. A good light from the window or from one of the daylight lanterns (figs. 46, 53) must traverse the lantern slide. This light is reflected up through the condenser by the plane mirror. The condenser will form a real image of the network or parallel lines at about the level where the object is placed on the slide. If now one focuses a 16 mm. (10x) or other objective upon this real image, it will appear very clearly in the field of the microscope. In order to utilize the image for micrometry the valuation of the spaces must be determined by the use of a stage micrometer as with the ocular micrometer (§ 375). Place a stage micrometer under the microscope and focus the lines sharply. Then with the screw or rack of the substage condenser focus the condenser up and down until the image of the lines or net on the lantern slide is also sharp. Arrange the stage micrometer so that the lines are parallel with the lines of the condenser image. Make any two of the lines coincide. Count the number of spaces in the condenser image included between any two of the lines of the stage micrometer, and divide the value of the space in the stage micrometer by the number of spaces of the condenser image included. The quotient will represent the valuation of the spaces of the condenser image in millimeters. For example, suppose the stage micrometer is ruled in 0.1 mm. and that 12 spaces of the condenser image are included in 9 spaces of the stage micrometer; then each space of the condenser image has a valuation of 0.9 mm. ÷ 12 = 0.075 mm.

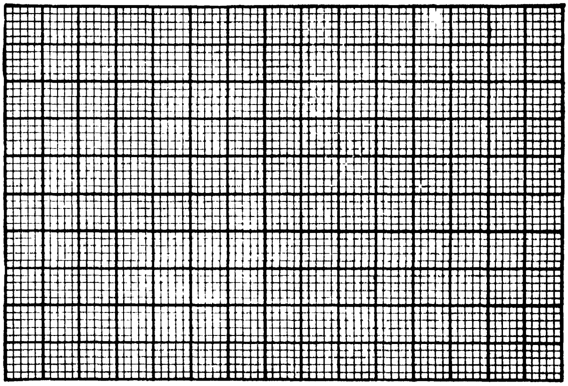


FIG. 164. NET SCALE FOR USE IN MICROMETRY WITH THE CONDENSER IMAGE.

As the size of the image varies with the distance of the object from the center of the condenser (§ 362), if the object (lantern slide of the lines) is always placed exactly the same distance in front of the microscope, the real image formed by the condenser will be of the same size, and hence have the same valuation for micrometry regardless of the power of the objective or the length of tube used. It is a very convenient method of micrometry for all coarser objects, but not exact enough for the finer objects. A movable scale or filar ocular micrometer should be used for the most exact work.

Example of an actual measurement by means of the condenser image: The long axis of a red corpuscle of necturus measured 0.61 of a space of the condenser image. As each space represents 0.075 mm. the length of the corpuscle is: 0.61 × 0.075 = 0.04575 mm. or 45.75μ. (See Chamot, pp. 155-157.)

§ **393. Remarks on micrometry.** — In using adjustable objectives (§§ 29, 149) the magnification of the objectives varies with the position of the adjusting collar, being greater when the adjustment is closed, as for thick cover-glasses, than when open, as for thin ones. This variation in the magnification of the objective produces a corresponding change in the magnification of the entire microscope and the ocular micrometer valuation; therefore it is necessary to determine the magnification and ocular micrometer valuation for each position of the adjusting collar.

While the principles of micrometry are simple, it is very difficult to get the exact size of microscopic objects. This is due to the lack of perfection and uniformity of micrometers and the difficulty of determining the exact limits of the object to be measured. Hence, all microscopic measurements are only approximately correct, the error lessening with the increasing perfection of the apparatus and the skill of the observer.

A difficulty when one is using high powers is the width of the lines of the micrometer. If the micrometer is perfectly accurate, half the width of each line belongs to the contiguous spaces, hence one should measure the image of the space from the centers of the lines bordering the space, or, as this is somewhat difficult in using the ocular micrometer, one may measure from the inside of one bordering line and from the outside of the other, that is, from the right side of all the lines, or from the left side of all. If the lines are of equal width this is as accurate as measuring from the center of the lines. Evidently it would not be right to measure from either the inside or the outside of both lines (figs. 157, 165).

It is also necessary in micrometry to use an objective of sufficient power to enable one to see all the details of an object with great distinctness. The necessity of using sufficient amplification in micrometry has been especially remarked upon by Richardson, Monthly Micr. Jour., 1874, 1875; Rogers, Proc. Amer. Soc. Microscopists, 1882, p. 239; Ewell, North Amer. Pract., 1890, pp. 97, 173.

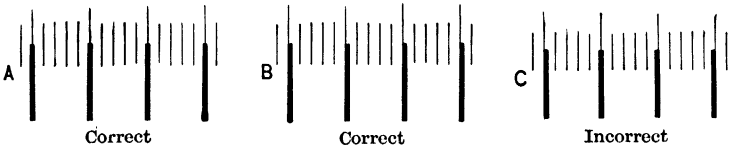


FIG. 165. CORRECT AND INCORRECT ARRANGEMENT OF THE OCULAR AND OF THE STAGE MICROMETER LINES.

(From Chamot).

The fine lines are those of the ocular micrometer and the coarse ones of the stage micrometer (compare fig. 157).

As to the limit of accuracy in micrometry, one who has justly earned the right to speak with authority expresses himself as follows: "I assume that 0.2μ is the limit of precision in microscopic measures beyond which it is impossible to go with certainty." W. A. Rogers, Proc. Amer. Soc. Micr., 1883, p. 198.

In comparing the methods of micrometry with the compound microscope given above (§§ 383–390), the one given in § 383 is impracticable; that given in §§ 388–390 is open to the objection that two standards are required — the stage micrometer and the steel rule; it is open to the further objection that several different operations are necessary, each operation adding to the probability of error. Theoretically the method given in § 385 is good, but it is open to the very serious objection in practice, that it requires so many operations which are especially likely to introduce errors. The method that experience has found most safe and expeditious, and applicable to all objects, is the method with the ocular micrometer. If the valuation of the ocular micrometer has been accurately determined, then the only difficulty is in deciding on the exact limits of the object to be measured and so arranging the ocular micrometer that these limits are enclosed by some divisions of the micrometer. Where the object is not exactly included by whole spaces on the ocular micrometer, the chance of error comes in, in estimating just how far into a space the object reaches on the side not in contact with one of the micrometer lines. If the ocular micrometer has some quite narrow spaces, and others considerably larger, one can nearly always manage to exactly include the object by some two lines. The ocular screw micrometers (figs. 158, 160) obviate this entirely, as the cross hair or lines traverse the object or its real image, and whether this distance be great or small it can be read off on the graduated wheel, and no estimation or guess work is necessary.

INDEPENDENT MAGNIFICATION OF OBJECTIVES AND OCULARS

§ 394. Independent magnification of an objective. — The independent magnification of an objective is like that of a projection microscope when the objective alone is used (figs. 147, 166). As pointed out in § 370 it is necessary to select some standard distance for the projection of the real or of the virtual image, for the size of the image varies directly as its distance from the center of the lens (fig. 152 for real and 145 for virtual images; in the latter the projection distance is from the nodal point in the eye to the image). The image distance for magnification most commonly employed is 250 mm. (§ 370).

While the magnification distance in microscopy has been fixed as 250 millimeters by general agreement, in actual use with a short or 160 mm. tube, the magnification of the objective is less than that which would be found by getting the magnification at the standard distance of 250 mm.

Now that the actual magnification produced by the objective on the short tube is used in designating it and this magnification number is correct no matter what kind of an ocular is used, it is worth while to know how it is obtained. In section eighteen the method is given. Briefly it is as follows: A stage micrometer is put upon the stage, and the objective to be used is put in place. A Huygenian ocular is inserted in the tube which has been set for a tube-length of exactly 160 millimeters (figs. 18, 26). The stage micrometer is then focused as sharply as possible. The Huygenian ocular is removed and a Ramsden micrometer ocular put in its place. Without focusing the microscope the least bit, the micrometer ocular is moved in the tube, or the tube is lengthened or shortened as necessary to give again a sharp image of the stage micrometer. The lines of the two micrometers are made parallel and the image of one or more spaces of the stage micrometer measured. Suppose the image of 0.10 mm. on the stage micrometer measures 1 mm., then the magnification of that objective with the short tube is 10, and this number is the one now marked on objectives of 16 mm. (10x) equivalent focus.

§ 395. Magnification due to the ocular. — The final magnification of the microscope (fig. 18) is due to the magnification of the objective multiplied by the magnification of the ocular. That is, the objective gives a real, magnified image, and the ocular as a whole gives a magnified image of the real image formed by the objective. The image formed by the ocular is measured at 250 millimeters distance, not at 160 millimeters as with the objective.

One of the best ways to determine the magnifying action of the ocular is to determine the magnification of the whole microscope (§§ 365, 367). Knowing the entire magnification, and knowing the magnification due to the objective, the part played by the ocular is the entire magnification divided by the objective magnification. For example, if the objective gives a magnification of 10, and the entire magnification of the microscope is 100 then $\frac{100}{10} = 10$, that is, the ocular must also have magnified 10.

If the ocular's magnification is 10 for a 250 mm. image distance, its equivalent focus must be $\frac{250}{10} = 25$, and the designation of this ocular would be 10x, or 25 e.f. and have an equivalent focus of 25 mm. It would magnify 10 diameters with any objective.

It may be puzzling to see how an objective with a magnification of 10, for example, could give the same final image with positive oculars, as with the negative oculars (figs. 24–25). The field lens serves to make the real image of the objective smaller (figs. 24–25) while the real image of the objective is formed below all the lenses of the positive oculars (figs. 22–23) and they all unite in acting as a magnifier.

The difficulty is overcome in this way: The curvature of the

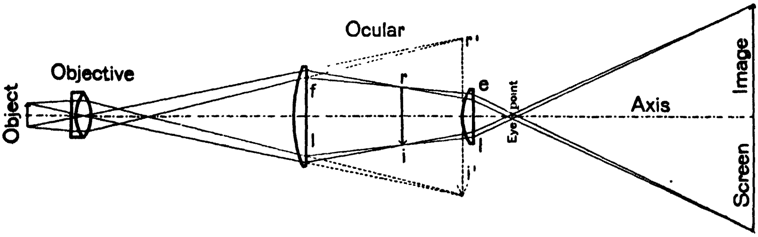


FIG. 166–167. MICROSCOPE MAGNIFICATION BY PROJECTION.

e l Eyelens of the ocular serving to project a real image to the screen.

f l Field lens, the lower lens in a negative ocular; it reduces the size of the real image formed by the objective.

r i Real image formed by the objective and field lens.

r' i' Position and size of the real image if no field lens were present.

The screen distance of 250 millimeters is measured along the axis from the eyepoint, not from the eyelens.

eyelens or combination as the case may be, is made enough greater to compensate for the reducing action of the field lens, and thus the ocular as a whole gives the desired increase in magnification, and its total action may be indicated with the same definiteness as with positive oculars, hence the magnification number on a Huygenian ocular while it gives no clue to the action of the individual lenses composing it, does indicate its final effect in producing the magnification of the microscope.

§ **396. Nelson's projection method of determining the magnification of the entire microscope.** — This method which has been rigidly tested by several observers and by myself side by side with the camera lucida method, gives such uniformly accurate results that it is recommended for general adoption. It is illustrated clearly by fig. 166. As used by the writer, the work was done by night or in a dimly lighted room.

The microscope is made horizontal and fastened to a block which slides on an optical bench (fig. 179). A dark-field lamp (figs. 79–80) is placed in line or at right angles to the microscope opposite the mirror (fig. 182).

A vertical white screen or a piece of finely ground glass is set up on a movable block beyond the ocular. The microscope is moderately lighted and the micrometer lines focused with extreme sharpness, then by means of a white card or piece of ground glass the position of the eyepoint of the ocular is determined, and the white vertical screen placed exactly 250 millimeters from the eyepoint. This is important. If the distance were measured from the top of the ocular, it would not give the correct result, and the error would be greater the higher the eye-point, as with the "telaugic" oculars (§§ 41, 145). The light in the microscope is now made as brilliant as possible, and the lines of the micrometer made as sharp as possible on the white screen by a slight turn of the fine adjustment.

With bow-dividers or other fine dividers the image of one or more spaces near the middle of the field is measured, and the spread of the dividers determined as in § 364. The total magnification can then be found by dividing the size of the image by the actual size of the micrometer space measured by the dividers (§ 367). (E. M. Nelson, Jour. Quekett Micr. Club, vol. xii, 1913, pp. 374-379.)

§ 397. Phelps Gage's method of obtaining the magnification for ocular and objective, and for the whole microscope. —

(1) A stage micrometer is used as object and focused sharply on the scale of an ocular micrometer in a positive ocular, both micrometers being in fractions of a millimeter.

(2) Make the microscope horizontal, light brilliantly with the 108-watt, 6-volt or other lamp. Determine the position of the eye-point of the ocular (§ 99). Put a vertical white screen 250 mm. from the eye-point and focus the stage micrometer on it. If the ocular micrometer is not also in focus on the screen, raise or lower the positive ocular combination until the lines are sharp. Refocus the stage micrometer if necessary.

(3) With bow or other fine dividers measure one or more spaces of each micrometer image.

(*a*) The image of the stage micrometer will represent the magnification of the entire microscope, objective and ocular.

(*b*) The image of the ocular micrometer will show the separate magnification of the positive ocular.

(*c*) The magnification of the whole microscope divided by the ocular magnification will represent the magnification of the objective on the 160 mm. tube.

If one knows the magnification of the objective, the magnification of any ocular, positive or negative, can be found by dividing the magnification of the whole microscope obtained as in 3*a* by that of the objective.

As the field lens of the negative ocular reduces the magnification of the objective, one can find the amount of the reduction by getting first the magnification of the whole microscope with the field lens in place and then with the field lens removed. As the eye-point is higher when the field lens is removed, one must readjust the microscope or the image screen to make the screen distance 250 mm. from the eye-point when getting the magnification.

§ 398. Magnification of drawings. — In determining the magnification of a drawing made with a camera lucida or with projection-apparatus, by far the best method is to remove the specimen and put in its place a stage micrometer and project the image of the micrometer upon the drawing paper. Make a few lines of the micrometer image and indicate the value of the spaces (fig. 172) then at any time one can determine exactly what the magnification is (§ 409).

COLLATERAL READING FOR CHAPTER VII

Sir A. E. Wright's Principles of Microscopy. Chamot, Chemical Microscopy, Chamot & Mason.

For those especially interested in micrometry in its relation to medical jurisprudence the following are recommended. They treat the subject in a practical as well as in a scientific spirit. The papers of Prof. Wm. A. Rogers on micrometers and micrometry, in the Amer. Quar. Micr. Jour., Vol. I. pp. 97, 208; Proceedings Amer. Soc. Microscopists, 1882, 1883, 1887. Dr. M. D. Ewell, Proc. Amer. Soc. Micr., 1890; The Microscope, 1889, pp. 43–45; North Amer. Pract. 1890, pp. 97, 173. Dr. J. J. Woodward, Amer. Jour. of the Med. Sci., 1875. M. C. White, Article "Blood Stains," Ref. Hand-Book Med. Sciences, 1885. Medico-Legal Journal, Vol. XII. For the change in magnification due to a change in the adjustment of adjustable objectives, see Jour. Roy. Micr. Soc. 1880, p. 702; Amer. Monthly Micr. Jour., 1880, p. 67. Carpenter-Dallinger, p. 270 and end of § 196.

If one consults the medico-legal journals, the microscopical journals, the Index Medicus, the Index Catalog of the library of the Surgeon General's Office, and The Quarterly Cumulative Index Medicus under Micrometry, Blood, and Jurisprudence, he can get on track of the main work which has been and is being done in legal medicine.

Optic Projection, S. H. & H. P. Gage.

Microscopy, E. J. Spitta.

The Microscope and its Revelations, Carpenter-Dallinger.

Journal of the Royal Microscopical Society.

Transactions of the American Microscopical Society, especially the address of Hon. J. D. Cox, 1884, pp. 5–39 on Aperture, and 1893, pp. 1–16, and A. C. Mercer, 1896, pp. 321–396.

John C. Shedd, The Index of Refraction. School Science and Mathematics, Vol. VI, 1906, pp. 678–680.

(This article gives a brief history of the discovery of the law of refraction; it also discusses the ratio of velocities in different media, and shows that the coefficient of retardation of velocity in a transparent medium is the reciprocal of the index of refraction.)

According to Nelson, "Par-focal" oculars have been made by Powell since 1839.

NELSON, E. M. — Eyepieces for the Microscope. Jour. Roy. Micr. Soc., 1908, p. 149. See also for other discussions of oculars by Nelson, same journal, 1907,

BECK, CONRAD. — The Microscope; Theory and Practice, London, 1938, pp. 45–47, 225–226.

CHAPTER IX

DRAWING WITH THE MICROSCOPE AND WITH PROJECTION APPARATUS; CLASS DEMONSTRATIONS

§§ 399–450; FIGURES 168–199

§ 399. Methods of drawing. — There are five principal methods for obtaining drawings in general, and all the methods are applicable to the production of drawings of microscopic objects:

(1) Free-hand drawings. This is the simplest method if one has natural ability and adequate training, for one needs only an object, pencil, pen and paper.

(2) Camera lucida drawings. By this method the outlines and proportions can be accurately traced (§§ 401–408).

(3) Camera obscura drawings. By this method the real image obtained in a photographic camera can be traced (§ 410).

(4) Projection drawings. In this method real images like those of the magic lantern and projection microscope can be traced directly upon the drawing paper (§ 418).

(5) Line drawings on blue prints and on the back of photographs (§§ 413–414).

In many laboratories all the methods are used, sometimes separately, but more often combined.

§ 400. Free-hand drawings. — Microscopic objects may be drawn free-hand directly from the microscope, but in this way a picture giving only the general appearance and relations of parts is obtained. For pictures which shall have all the parts of the object in true proportions and relations, it is necessary to obtain an exact outline of the image of the object, and to locate in this outline all the principal details of structure. It is then possible to complete the picture free-hand from the appearance of the object under the microscope.

§ 401. Camera lucida. — This is an optical apparatus for enabling one to see objects in greatly different situations as if in one field of vision, and with the same eye. In other words, it is an optical device for superimposing or combining two fields of view in one eye.

As applied to the microscope, it causes the magnified virtual image of the object under the microscope to appear as if projected upon the table or drawing board, where it is visible with the drawing paper, pencil, dividers, etc., by the same eye, and in the same field of vision. The microscopic image appears like a picture on the drawing paper (§ 404a). This is accomplished in two distinct ways:

(1) By a camera lucida reflecting the rays from the microscope so that their direction when they reach the eye coincides with that of the rays from the drawing paper, pencil, etc. In some of the camera lucidas from this group (Wollaston's, fig. 168), the rays are reflected twice, and the image appears as when looking directly into the microscope. In others the rays are reflected but once, and the image has the inversion produced by a plane mirror. For drawing purposes this inversion is a great objection, as it is necessary to invert similarly all the details added freehand.

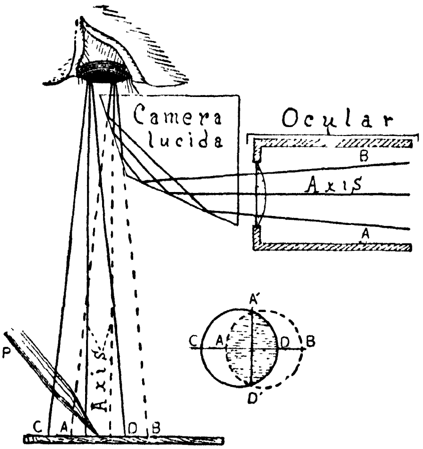


FIG. 168. WOLLASTON'S CAMERA LUCIDA.

Axis The optic axis of the microscope.

Ocular The upper end of the ocular.

A, *B* Two rays outside the axis to show that they cross twice and hence have the same relative position as when they emerge from the ocular.

Camera lucida The quadrangular piece of glass giving the double internal reflection to change the direction of the axial ray 90°.

CD, *AB* The virtual image, drawing paper and pencil partly overlapping. Where they overlap the appearance is that of one field.

(2) By a camera lucida reflecting the rays of light from the drawing paper, etc., so that their direction when they reach the eye coincides with the direction of the rays from the microscope (fig. 169). In all of the camera lucidas of this group, the rays from the paper are twice reflected and no inversion appears.

The better forms of camera lucidas (Wollaston's, Grunow's, Abbe's, etc.) may be used for drawing both with low and with high powers. Some require the microscope to be inclined (fig. 168) while others are designed to be used on the microscope in a vertical position. As in biological work it is often necessary to have the microscope vertical, the form for a vertical microscope is to be preferred (fig. 169).

§ **402. Avoidance of distortion.** — In order that the picture drawn by the aid of a camera lucida may not be distorted, it is necessary that the axial ray from the image on the drawing surface shall be at right angles to the drawing surface (figs. 168, 170).

§ **403. Wollaston's camera lucida.** — This is a quadrangular prism of glass put in the path of the rays from the microscope, and it serves to change the direction of the axial ray 90 degrees. In using it the microscope is made horizontal, and the rays from the microscope enter one-half of the pupil, while rays from the drawing surface enter the other half of the pupil. As seen in fig. 168, the fields partly overlap, and where they do so overlap, pencil or dividers and microscopic image can be seen together.

In drawing or using the dividers with the Wollaston camera lucida it is necessary to have the field of the microscope and the drawing surface about equally lighted. If the drawing surface is too brilliantly lighted, the pencil or dividers may be seen very clearly, but the microscopic image will be obscure. On the other hand, if the field of the microscope has too much light, the microscopic image will be very definite, but the pencil or dividers will not be clearly visible. It is necessary, as with the Abbe camera lucida (§ 404), to have the Wollaston prism properly arranged with reference to the axis of the microscope and the eyepoint. If it is not, one will be unable to see the image well, and may be entirely unable to see the pencil and the image at the same time. Again, as rays from the microscope and from the drawing surface must enter independent parts of the pupil of the same eye, one must hold the eye so that the pupil is partly over the camera lucida and partly over the drawing surface. One can tell the proper position by trial. This is not a very satisfactory camera to draw with, but it is a very good form to measure the vertical distance of 250 mm. at which the drawing surface should be placed when determining magnification (fig. 153).

§ **404. Abbe camera lucida.** — This consists of a cube of glass cut into two triangular prisms and silvered on the cut surface of the upper one. A small oval hole is then cut out of the center of the

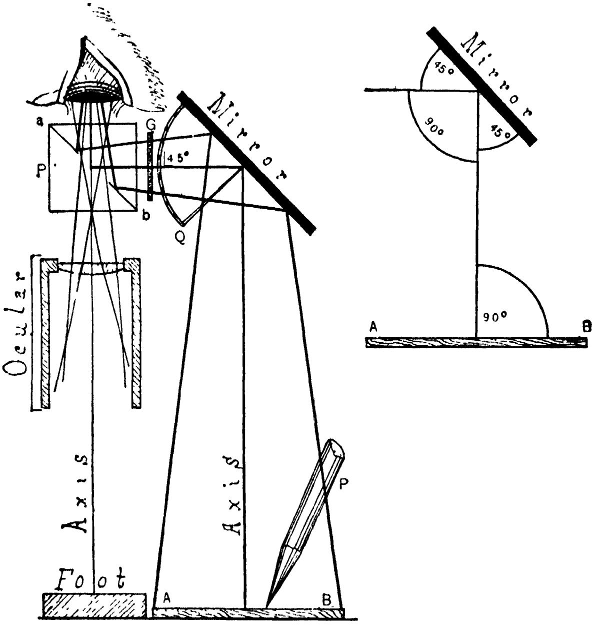


Fig. 169. Diagram of Abbe's Camera Lucida with a Vertical Microscope.

Axis, *Axis* The axial ray of the microscope and from the field of the drawing surface.

Ocular The upper part of the microscope ocular.

Mirror The mirror of the camera lucida reflecting the rays from the drawing surface at right angles to the axis.

P, *P* The drawing pencil in the field, and the prism of the camera lucida.

Q The quadrant attached to the mirror to give the angle.

G Smoked glass.

a b The silvered surface in the prism with a hole made in the center for the light to pass upward from the microscope. The silvered part reflects the rays from the drawing surface.

The geometrical figure at the left gives the angles when a 45° mirror is used.

silvered surface and the two prisms are cemented together in the form of the original cube with a perforated 45 degree mirror within it (figs. 169–170). The upper surface of the cube is covered by a perforated metal plate. This cube is placed over the ocular in such a way that the light from the microscope passes through the hole in the silvered face and thence directly to the eye. Light from the drawing surface is reflected by the mirror to the silvered surface of the prism and reflected by this surface to the eye in company with the rays from the microscope, so that the two fields appear as one, and the image is seen as if on the drawing surface (figs. 168–171, § 404a).

§ **404a.** For some persons the image and the drawing surface, pencil, etc., do not appear on the drawing board as stated above, but under the microscope, according to the general principle that "objects appear in space where they could be touched along a perpendicular to the retinal surface stimulated," — that is, in the line of rays entering the eye. This is always the case with the Wollaston camera lucida. The explanation of the apparent location of the image, etc., on the drawing board with the Abbe camera lucida is that the attention is concentrated upon the drawing surface rather than upon the object under the microscope. With some observers it is possible to make the image appear under the microscope or on the drawing surface at will by concentrating the attention of one position or the other. (Dr. W. B. Pillsbury).

§ **405. Arrangement of the camera lucida prism.** — In placing this camera lucida over the ocular for drawing or for the determination of magnification, the center of the hole in the silvered surface is placed in the optic axis of the microscope. This is done by properly arranging the centering screws that clamp the camera to the microscope tube or ocular. The prism must not only be centered to the axis of the microscope, but it must be at the right level, or more or less of the field will be cut off. In all the good modern forms of this camera lucida it is fastened to the tube of the microscope by a clamp which enables one to raise or lower it so that it may be at the right position with reference to the eyepoint of the ocular being used (§ 99).

One can determine when the camera is in a proper position by looking into the microscope through it. If the field of the microscope appears as a circle and of about the same size as without the camera lucida, then the prism is in a proper position. If one side of the field is dark, then the prism is to one side of the center; if the field is considerably smaller than when the prism is turned off the ocular, it indicates that it is not at the correct level, i.e., it is above or too far below the eyepoint.

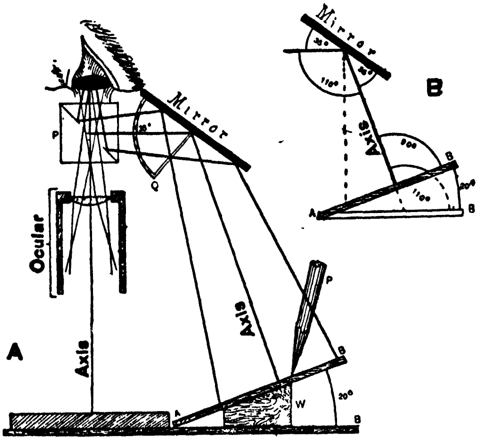


FIG. 170. DIAGRAM OF THE ABBE CAMERA LUCIDA WITH THE DRAWING SURFACE ELEVATED TO MAKE THE AXIS PERPENDICULAR WITH DEPRESSED MIRROR

A, *Axis*, *Axis* The axial ray from the microscope and from the drawing surface.

Ocular The upper part of the microscopic ocular.

Mirror The mirror of the camera lucida; it is depressed from 45° to 35° to make the axis from the drawing surface perpendicular to the axis of the microscope.

A — *B* The drawing surface elevated 20°; that is, twice as many as the mirror is depressed below 45°.

W Wedge under the drawing board.

P, *P* The drawing pencil and the prism of the camera lucida.

Q Quadrant of the mirror.

B Geometrical figure to show why the drawing board must be raised twice as many degrees as the mirror is depressed to keep the axial ray perpendicular to the drawing surface.

§ 406. Arrangement of the mirror and the drawing surface. — The Abbe camera lucida was designed for use with a vertical microscope (fig. 169). On a vertical microscope if the mirror is set at an angle of 45°, the axial ray is at right angles with the table top or drawing board which is horizontal, and a drawing made under these conditions is in true proportion and not distorted. The stage of most microscopes, however, extends out so far at the sides that with a 45° mirror the image appears in part on the stage of the microscope. In order to avoid this, the mirror may be depressed to some point below 45°, say at 40° or 35° (fig. 170). But as the axial ray from the mirror to the prism must still be reflected horizontally, it follows that the axial ray no longer forms an angle of 90° with the drawing surface, but a greater angle. If the mirror is depressed to 35°, then the axial ray makes an angle of 110° with a horizontal drawing surface (fig 170 *B*). To make the angle 90° again, so that there shall be no distortion, the drawing board must be raised toward the microscope 20°. The general rule is to raise the drawing board twice as many degrees toward the microscope as the mirror is depressed below 45°. Practically, the field for drawing can always be made free of the stage of the microscope, at 45°, at 40°, or at 35°. In the first case (45° mirror) the drawing surface should be horizontal, in the second case (40° mirror) the drawing face should be elevated 10°, and in the third case (35° mirror) the drawing board should be elevated 20° toward the microscope. Furthermore, it is necessary in using an elevated drawing board to have the mirror bar of the camera lucida project directly laterally so that the edges of the mirror are in planes parallel with the edges of the drawing board; otherwise there will be front to back distortion, although the elevation of the drawing board avoids right to left distortion. If one has a micrometer ruled in squares (net micrometer) (figs. 131, 164), the distortion produced by not having the axial ray at right angles with the drawing surface may be very strikingly shown. For example, set the mirror at 35° and use a horizontal drawing board. With a pencil make dots at the corners of some of the squares, and then with a straight edge connect the dots. The figures will be considerably longer from right to left than from front to back. Circles in the object appear as ellipses in the drawings, the major axis being from right to left.

The angle of the mirror may be determined with a protractor, but that is troublesome. It is much more satisfactory to have a quadrant attached to the mirror and an indicator on the projecting arm of the mirror. If the quadrant is graduated throughout its entire extent, or preferably at three points, 45°, 40° and 35°, one can set the mirror at a known angle in a moment; then the drawing board can be hinged and the elevation of 10° and 20° determined with a protractor. The drawing board is very conveniently held up by a broad wedge. By marking the position of the wedge for 10° and 20° the protractor need be used but once; then the wedge may be put into position at any time for the proper elevation.

§ **407. Abbe camera and inclined microscope.** — It is very fatiguing to draw continuously with a vertical microscope, and many mounted objects admit of an inclination of the microscope, when one can sit and work in a more comfortable position. The Abbe camera is as perfectly adapted to use with an inclined as with a vertical microscope. All that is requisite is to be sure that the fundamental law is observed regarding the axial ray of the image and the drawing surface, viz.. that they should be at right angles. This is very easily accomplished as follows: The drawing board is raised toward the microscope twice as many degrees as the mirror is depressed below 45° (§ 406); then it is raised exactly as many degrees as the microscope is inclined, and in the same direction, that is, so that the end of the drawing board shall be in a plane parallel with the stage of

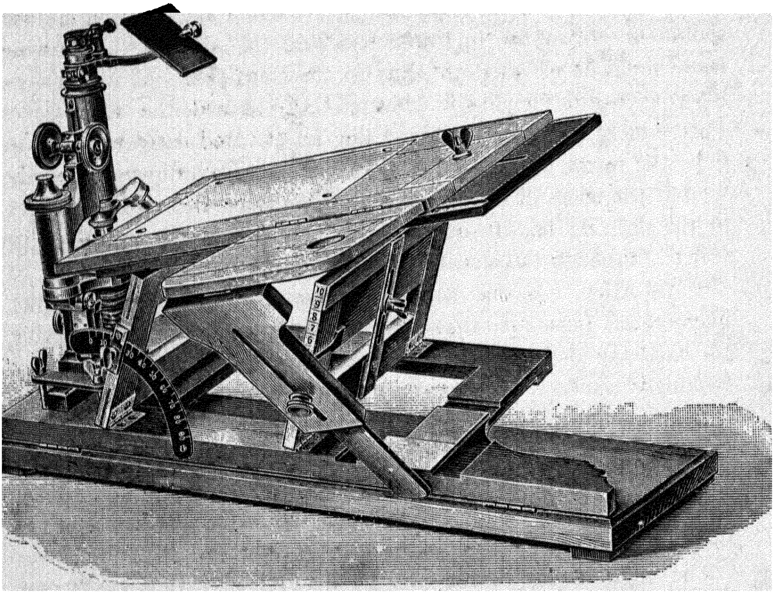


FIG. 171. BERNHARD'S DRAWING BOARD FOR THE ABBE CAMERA LUCIDA. (From the Catalogue of Zeiss).

This drawing board can be elevated and tipped; it can also be inclined, carrying the microscope with it.

the microscope. The mirror must have its edges in planes parallel with the edges of the drawing board also (fig. 171).

§ **408. Drawing with the Abbe camera lucida.** — (1) The light from the microscope and from the drawing surface should be of nearly equal intensity, so that the image and the drawing pencil can be seen with about equal distinctness. This may be accomplished with very low powers (16 mm. (10x) and lower objectives) by covering the mirror of the microscope with white paper when transparent objects are to be drawn. For high powers it is best to use a substage condenser. Often the light may be balanced by using a larger or smaller opening in the diaphragm. One can tell which field is excessively illuminated, for it is the one in which objects are most distinctly seen. If it is the microscopic, then the image of the microscopic object is very distinct and the pencil is invisible or very indistinct. If the drawing surface is too brilliantly lighted, the pencil can be seen clearly, but the microscopic image is obscure.

When opaque objects, that is, objects which must be lighted with reflected light (figs. 19, 43), like dark colored insects, etc., are to be drawn, the light must usually be concentrated upon the object in some way. The microscope may be placed in a very strong light and the drawing board shaded, or the light may be concentrated upon the object by means of a concave mirror, or a bull's-eye condenser or the small arc lamp may be used.

If the drawing surface is too brilliantly illuminated, it may be shaded by placing a book or a ground-glass screen between it and the window, also by putting one or more smoked glasses in the path of the rays from the mirror (fig. 169). If the light in the microscope is too intense, it may be lessened by using white paper over the mirror, or by a ground-glass screen between the microscope mirror and the source of light (Piersol, American Monthly Microscopical Journal, 1888, p. 103). It is also an excellent plan to blacken the end of the drawing pencil with carbon ink. Sometimes it is easier to draw on a black surface, using a white pencil or style. The carbon paper used in manifolding letters, etc., may be used, or ordinary black paper may be lightly rubbed on one side with a moderately soft lead pencil. Place the black paper over white paper and trace the outlines with a pointed style of ivory or bone. A corresponding dark line will appear on the white paper beneath (Jour. Roy. Micr. Soc., 1883, p. 423).

(1) It is desirable to have the drawing paper fastened with thumb tacks, or in some other way. (2) The lines made while using the camera lucida should be very light, as they are likely to be irregular. (3) Only outlines are drawn and parts located with a camera lucida. Details are put in free-hand. (4) It is sometimes desirable to draw the outline of an object with a moderate power and add the details with a higher power. If this is done, it should always be clearly stated. It is advisable to do this only with objects in which the same structure is many times duplicated, as in a nerve or a muscle. In such an object all the different structures can be shown, and by omitting some of the fibers the others may be made plainer without undesirable enlargement of the entire figure. (5) If a drawing of a given size is desired and it cannot be obtained by any combination of oculars, objectives, and lengths of the tube of the microscope, the distance between the camera lucida and the table may be increased or diminished until the image is of the desired size. This distance is easily changed by the use of a book or a block, but more conveniently if one has a drawing board with adjustable drawing surface like that shown in fig. 171. (6) It is of advantage to have the camera lucida hinged so that the prism may be turned off the ocular for a moment's glance at the preparation, and then returned without the necessity of loosening screws and readjusting the camera. This form is now made by several opticians, and many of them add graduations so that the angle of the mirror is readily seen.

§ **409. Scale of drawings.** — The scale should be given for every drawing (fig. 172). Sometimes the drawing is larger than the object, as with microscopic specimens, and sometimes it is of the same size or much smaller, as in drawing large objects.

In getting the scale at which an object is drawn with the microscope or projection microscope, the object is removed and a micrometer in half millimeters for low powers and one in tenths and hundredths of a millimeter (fig. 148) for high powers is put in place of the specimen. The image of the micrometer lines and spaces will be of the same enlargement as the drawing, provided nothing has been changed except the micrometer for the object. If now a few of the lines of the micrometer image (figs. 148, 172) are traced at one corner of the drawing paper and their actual value given, the enlargement can be determined accurately as follows: Suppose the micrometer spaces are tenth millimeters, and the image of the spaces measures 2 millimeters. The enlargement must be the size of the image divided by the size of the object or 2 ÷ 0.1 = 20; that is, the image is 20 times the size of the object.

In using the photographic camera for negatives or for tracing, if the metric scale (fig. 173) is put with the object, its image will appear with the image in the negative or in the tracing, and the enlargement or reduction can be found as above. Suppose the image of the 10 cm. scale on the negative or in the tracing is 2 cm. long. Obviously the picture must be 2 cm. ÷ 10 = $\frac{2}{10}$ or $\frac{1}{5}$, that is, the picture is only one-fifth the size of the object.

For any form of projection apparatus (figs. 178–183), the magic lantern or projection microscope, after the image is traced, the object is removed and a micrometer in half millimeters for the magic lantern and low powers of the microscope is put in place of the object and the image of the scale projected upon the drawing paper. Suppose the image of one of the micrometer half millimeter spaces measures 15 millimeters, then the scale of the drawing must be 30 (i.e., 15 ÷ $\frac{1}{2}$ = 30).

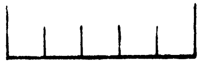


FIG. 172. MAGNIFIED MICROMETER SPACES TO SHOW THE METHOD OF INDICATING THE SCALE AT WHICH DRAWING WAS MADE.

If one is drawing from the projected image of a negative or lantern slide it is necessary to know the scale at which the negative or slide was made as well as the scale at which the drawing from the projected negative or slide is being made. For example, if the scale of the negative is 50 times the size of the object, and the drawing is 10 times the size of the negative, the final drawing must be 10 × 50 = 500 times the size of the original object.

If, on the other hand, the negative is $\frac{1}{10}$ the size of the original object and the drawing is 5 times the size of the negative, the final drawing will be the size of the negative ($\frac{1}{10}$ the original) multiplied by the magnification (in this case 5) which is $\frac{1}{10} \times 5 = \frac{5}{10}$ or $\frac{1}{2}$. That is, the drawing is one-half the size of the original object.

For the projection microscope with objectives of 40 to 16 mm. (4x to 10x) a micrometer in $\frac{1}{2}$ mm. is good. For objectives above 16 mm. (10x) it is better to use a micrometer in 0.1 mm. and 0.01 mm. (fig. 148).

After the drawing has been made, remove the specimen and put the micrometer under the microscope and draw a few spaces of the micrometer image (fig. 172) giving the actual value of the spaces; then one can compute the enlargement of the drawing by measuring the image spaces and dividing by the actual value. For example, suppose the image of one of the 0.1 mm. spaces measures on the drawing 4 cm. or 40 mm. The scale of the drawing or its magnification is 40 ÷ 0.1 = 400.

§ **409a.** For diagrams and other large objects a very serviceable micrometer can be made by using the 10 cm. metric rule (fig. 173) as object and making a negative of it on a lantern slide exactly natural size or half natural size.

10 CENTIMETER RULE

The upper edge is in millimeters, the lower in centimeters

The metric system

UNITS.	*The most commonly used divisions and multiples.*
THE METER FOR LENGTH	*Centimeter* (cm.) 0.01 Meter; *Millimeter* (mm.), 0.001 Meter; *Micron* (μ), 0.001 Millimeter; the Micron is the unit in Micrometry. *Kilometer*, 1000 Meters; used in measuring roads and other long distances.
THE GRAM FOR WEIGHT	*Milligram* (mg.), 0.001 Gram. *Kilogram*, 1000 Grams, used for ordinary masses, like groceries, etc.
THE LITER FOR CAPACITY,	*Cubic Centimeter*, (cc.), 0.001 Liter. This is more common than the correct form, Milliliter.

Divisions of the *Units* are indicated by the Latin prefixes; *deci.* 0.1; *centi*, 0.01; *milli*, 0.001; *micro*, one millionth (0.000001) of any unit.

Multiples are designated by the Greek prefixes; *deka*, 10 times; *hecto*, 100 times; *kilo*, 1000 times; *myria*, 10,000 times; *mega*, one million (1,000,000) times any unit.

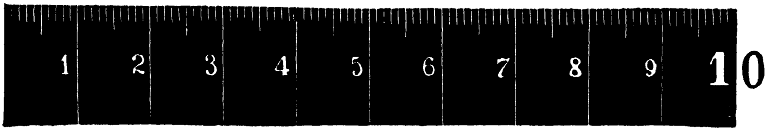


FIG. 173. METRIC SCALE AND SUMMARY OF THE METRIC SYSTEM.

DRAWINGS BY THE AID OF THE PHOTOGRAPHIC CAMERA AND THE MAGIC LANTERN

§ **410. Drawings by the aid of a photographic camera.** — The photographic camera (camera obscura) gives help for getting pictures of objects in three ways:

(1) By producing real images which can be traced (§ 411).

(2) By producing negatives which can be projected upon the drawing paper and traced, or the drawing can be done directly on the print, and all but the drawing removed from the print; or the drawing can be made on the back of the print (§§ 413–414).

(3) By producing large prints for retouching (§ 416).

411. Real images by the camera. — For drawing with a photographic camera it is a great help to have a frame with a piece of clear glass to use instead of the ordinary ground-glass focusing screen. The tracing paper is stretched over the glass. The object is arranged as desired and placed in a strong light. The camera is then arranged to give the desired view, and the bellows pulled out, and the whole camera moved toward or away from the object until the desired size is obtained. This tracing is transferred to the drawing paper in the usual manner and inked in. A camera like that shown in fig. 174 answers well; also a copying camera.

While inking in, and indeed whenever free-hand and optical methods of getting drawings are combined, the object should be available for constant observation so that accuracy may be obtained.

§ **412. Negatives by the camera.** — The object is arranged as desired and placed in a good light. A photographic camera is then used and a negative on glass made in the usual manner. If the negative is to be used for prints on which to trace and draw with ink or pencil, the negative is made the size of the desired finished picture. On the other hand if the negative is to be used for projection, it should be of about the size of a lantern slide (§ 416).

§ **413. Drawings upon blue prints.** — This is especially available for objects with definite outlines and clear details like the wing veins of insects (Comstock) or apparatus, furniture, etc.

A negative of the object is made of the desired size and a blue print made. Then with waterproof India ink all the lines are gone over, and all the points indicated which are to be shown in the finished cut.

Bleach out the blue by soaking the print in a solution of 10%

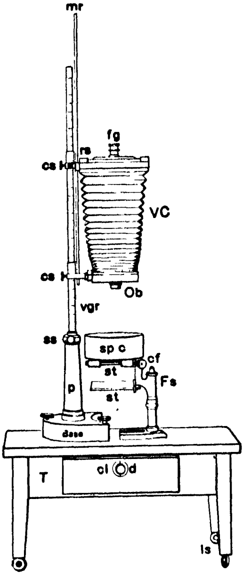


FIG. 174. VERTICAL PHOTOGRAPHIC CAMERA ON A LOW TABLE.

T Table about 50 cm. high and 50 cm. by 70 cm. on the top.
cl d Drawer with combination lock.
Base The heavy base of the vertical camera support.
p Pillar in which the graduated rod (*vgr*) rotates.
ss Set screw to fix the graduated rod in any position.
c s, *c s* Set screws to enable the operator to set the camera bellows at any desired extension.
mr Magnification rod with its set screw *rs*. When any desired magnification is arranged, the rod set screw is tightened; then by loosening the camera set screws (*cs*) the bellows can be moved up and down on the graduated rod to get the focus.
Fs Focusing stand; this is a microscope stand with coarse and fine adjustment (*cf*) and two stages (*st st*) for supporting the object or the dish containing it (*sp c*).
Ob Photographic objective in the lower end of the camera.
VC Vertical camera bellows.
fg Focusing glass.

neutral oxalate of potash. Wash in water and dry on gauze. Only the ink lines will show in the finished print. This line drawing can then be lettered in any desired way, and the engraver can make a line cut for the printing press.

Ordinarily it is best to make the picture two or three times the size of the final engraving. Defects are minimized in the reduction. Always have the object in view in finishing the drawing.

§ **414. Drawings on the back of photographic prints.** — The easiest way to obtain line cuts of many objects is to make a photograph of them and then draw the outlines on the back of the photograph. This is an application of the old method of tracing the veins of leaves and the details of other objects by holding against a well lighted window, and making the tracing on a sheet of paper over the object.

A negative should be made of the size desired or a small negative is made and a large print obtained by projection (§ 484). Prints of both sides of the negative should be made. A print from the front or film side will give an erect image like the object. One from the glass side or back of the negative will give a reversed image. The tracing on the back of the reversed or inverted image will give an erect image like the object.

If one prints by projection (§ 484), the erect image is made by making the negative, film side, face the printing paper; the inverted image is obtained by having the glass side of the negative face the paper.

If the original negative is of the desired size, one print is made by putting the sensitive paper in contact with the front or film side of the negative. For the inverted or reversed print the glass side is placed up in the printing frame, and the sensitive paper put on the glass. This will make the print slightly out of focus, but by printing this image with the plate holder a meter or more distant, and directly under the printing light, a moderately sharp print can be made except for very thick glass negatives. There will be no trouble with films.

§ **415. Making the tracings.** — When the enlarged prints are ready, proceed as follows: Work in a dark or dimly lighted place.

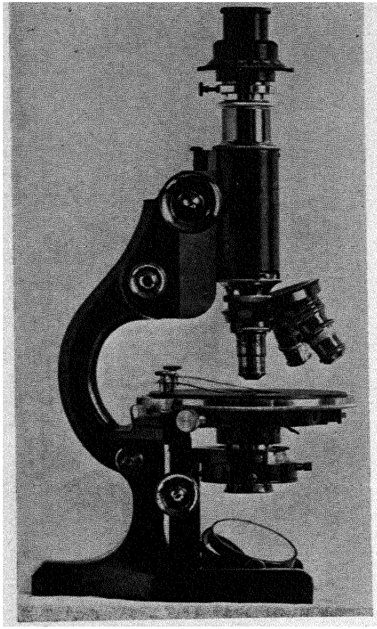


FIG. 175

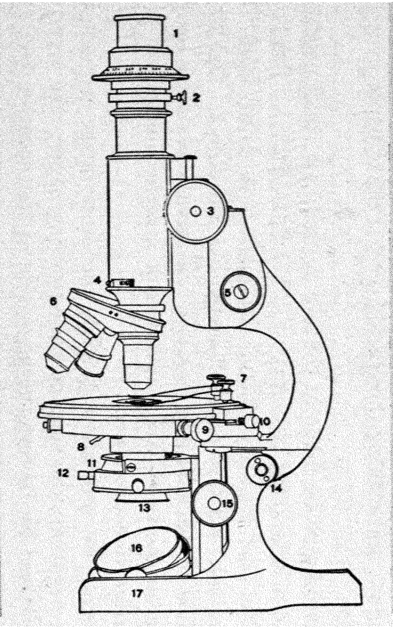


FIG. 176

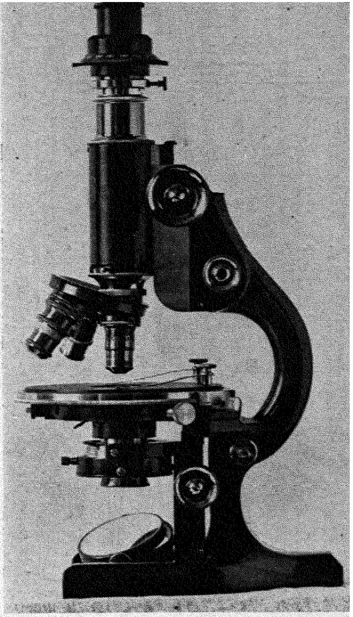


FIG. 177

ILLUSTRATIONS TO SHOW THE METHOD OF TRACING OUTLINES ON THE BACK OF PHOTOGRAPHIC PRINTS.

Fig. 175 is an inverted print, and 177 a non-inverted one. The outline shown in fig. 176 was traced on the back of fig. 175. This made it erect like fig. 177.

Use a drawing shelf containing a glass window (fig. 180), or use a table with a heavy glass set in a window in the top. Have a 100-watt lamp in a reflector underneath to illuminate the print.

Place the inverted print, face down, on the glass. The light shining through the print will make it appear almost as if the face were up.

Trace all the outlines with a lead pencil, using a triangle or T-square for the straight lines. In doing the tracing it is advantageous to have the erect print to look at, and occasionally one should hold the tracing in a good light to see that all the lines are present.

After getting the outlines with a pencil, the lines are inked in. For this one should work in a well-lighted place, and have the actual object in view and the erect print to serve as guides. Some additions may be put in free-hand. One may also wish to add accessory apparatus, or enlargements of some of the details. This was done with figures 80 and 180.

The paper used for photographic prints is excellent both for the draughtsman and for the photo-engraver. There is some advantage in using double-thick paper for the tracings, as the prints are flatter. The single thickness of paper shows the details of the print somewhat more clearly.

Of course, one could make tracings on the back of blue prints, and then no bleaching would be necessary, but the details are not so sharp and definite in blue prints as in silver prints. One can draw on the face of silver prints and remove the silver print with chemicals, but that is not so satisfactory as drawing on the back of a reversed print as described above.

Many of the line drawings in this book were made by tracing them on the back of inverted photographic prints. Much has also been made of the method for all sorts of objects during the last ten years, and its usefulness is increasingly appreciated. The amateur artist has the advantage of correct proportions and perspective without the trouble of many measurements; he is also perfectly free to add artistic touches, and to combine free-hand sketches. The line cuts have the great advantage of definiteness, and can be printed on any good book paper. For lettering the drawings, see

§ 427. Do not make the lettering so prominent that the drawing itself is submerged.

§ 416. Retouching photographs for halftone reproduction. — For pictures of animals, organs, and dissections to be reproduced by the halftone process, very successful drawings can be made as follows: Arrange the object as it is to appear in the finished drawing; light it to bring out clearly the features desired; then use a long focus photographic objective and get a small, sharp picture. The negative should be about the size of a lantern slide. Make a large print on thick developing paper exactly as described in section 484. This print should not be dark, but two or three shades lighter than the usual print to give opportunity for the added shading. The picture should be erect.

When the print is dry, put it on a drawing board and with a carbon drawing crayon, pen, India ink, and an air brush, if it is available, the picture can be made almost perfect with a minimum of labor.

In case the negative shows parts not needed or if the background is not as desired, the superfluous parts can be eliminated and the background made perfectly white by painting on the glass surface of the negative Gihon's or other opaquing medium. In the print there will be pure white where the opaque is painted on the glass. Use a fine brush and put on a layer which does not allow any light to pass. The opaque is put on the glass surface so that it can be removed easily if desired. In case some parts are not light enough or white points are to be added, use some of the white recommended by the photo-engravers (Blanc d Argent, etc.).

As in all drawing, the actual object should be before the artist when retouching the photograph, so that accuracy may be secured.

§ 417. Tracing pictures natural size on drawing paper. — It frequently happens in preparing the drawings for a book or for a scientific paper that figures from another book or scientific paper are needed. If there is to be no modification in the figure, the simplest method is to borrow an electrotype. If this cannot be done and the picture is not available to put in the hands of the photo-engraver for a new cut, or if one wants to make minor changes, it is very easy to get a tracing on any good drawing paper as follows:

Put the picture on the glass of the drawing shelf (fig. 180) and place over it some good drawing paper like Whatman's hot-pressed drawing paper or Reynolds' bristol board. Turn on the light, and even through the thick drawing paper the outlines of the picture are so clear that the tracing can be made with ease. After the outlines have been traced, the finishing can be done on a drawing board with the original picture for reference.

§ **418. Diagrams by projection.** — For light use an arc lamp or a stereopticon mazda lamp; use a negative which is not too dense or a lantern slide. It is placed in the lantern-slide holder and by means of an ordinary projection objective, or better by a photographic objective, the image is projected upon the drawing paper (fig. 178). For the proper size either the projection apparatus or the drawing surface must be movable.

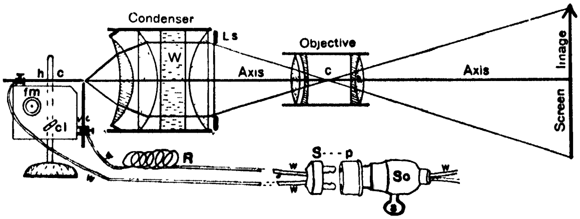


FIG. 178. MAGIC LANTERN WITH PROJECTED IMAGE.
(From Optic Projection).

A small arc lamp connected with the house lighting system is used for light in this case.

W, *So*, *S — p* Electric wires, lamp socket with key switch (*s*) and a separable attachment plug.

R Rheostat.

Condenser, *W* A three lens condenser with a water cell to absorb radiant heat.

LS Lantern slide.

Axis, *Objective* The principal optic axis of the condenser and of the objective in one line. The cone of light crosses within the objective at (*c*).

Screen Image The real image projected upon the screen.

When the size is correct, and the image sharply focused, one can trace directly on the drawing paper with a pencil all the lines and details which it is desired to represent. Then the drawing can be inked in at leisure, remembering always to have the object for constant reference and thus insure accuracy.

In projection it is very easy to make the picture as large as desired provided the projection apparatus or the drawing surface is movable. The projection method has the advantage of being applicable to all forms of objects, gross and microscopic. The only precaution is to make the negative rather thin, not dense; then the details come out clearly in the projected image.

PROJECTION MICROSCOPE FOR DRAWING

§ **419.** This is the most satisfactory method of drawing small objects. With it one can draw large diagrams or small figures directly from the objects; and if the apparatus is properly constructed one may make diagrams from objects 60 to 70 mm. in diameter down to those of half a millimeter or less. This method was much in vogue and was highly commended by the older microscopists who used the solar microscope (Baker, Adams, and Goring). Since the general introduction of electric lighting, drawing with the projection microscope has become once more common and is the most satisfactory method known, especially for the numerous drawings necessary for the preparation of models in wax or blotting paper.

§ **420. Drawings with low powers.** — For objectives of 30 to 100 mm. focus, the best method is to use a projection outfit with a three lens condenser as shown in fig. 179.

For a radiant, a large or a small arc lamp is best (figs. 179, 181), but a 250- or 400-watt concentrated filament, stereopticon mazda lamp filled with nitrogen also works fairly well. The mazda lamp has the advantage that it can be attached to any lighting circuit, and when once centered and properly arranged, requires no attention except to turn the switch on and off. A dark room is desirable, but one can draw in any room at night.

Arrange the object, the lamp, and the condenser so that the object is fully lighted; then focus the objective and place the drawing surface and objective at a distance apart to give the desired size of drawing. Focus sharply and trace with a pencil the outlines and details which it is desired to show. Finally, with the object where it can be examined at any time, ink in the lines and details. (For erect images see § 435).

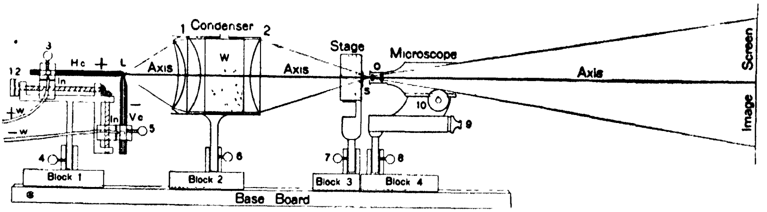


FIG 179. PROJECTION MICROSCOPE.

(From Optic Projection).

+w The positive wire going to the upper carbon (*Hc*), and *−w*, wire to the lower or vertical carbon (*Vc*) of the large arc lamp with direct current.

Axis, *Axis*, *Axis* The principal optic axis from the source of light (*L*) through the condenser, the microscope and to the screen.

W Water cell to absorb radiant heat.

Stage The separate stage of the microscope with its water cell for cooling the specimen by conduction.

Microscope In this case the microscope has an objective only; compare fig. 180, where an ocular is present also.

Each element, lamp, condenser, stage, and microscope is on a separate movable block (block 1, 2, 3, 4) which slides independently along the optic bench or base board.

§ **421. Use of a 45° mirror or a prism.** — While one can draw on a vertical surface, it is far easier to draw on a horizontal surface. This is available for all powers by using a plane mirror at 45° or a drawing prism. The mirror may be at a distance from the objective, when it must be large (fig. 181); or it may be close to the objective, when it may be small (figs. 180, 183). The drawing surface must be movable to vary the size of the drawing and the magnification. Figures 179–181 show the two principal methods of varying the distance between the objective and the drawing surface, and consequently the scale of the drawing. (For erect images see §§ 430–437.)

§ **422. Drawing with objectives of 25 (5x) to 8mm. (20x) focus.** — For this the best way is to use a three lens condenser, as shown in figs. 179, 180, and for a microscope use either the special one for projection or the ordinary microscope with large tube. For radiant use a small or a large arc lamp. Remove the substage condenser or turn it aside and arrange on the optical bench so that the image

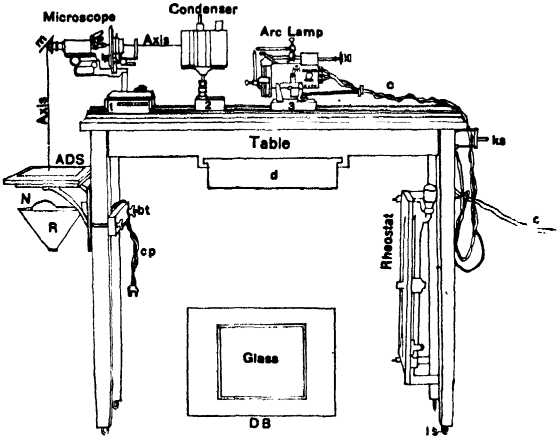


FIG. 180. PROJECTION MICROSCOPE, TABLE, AND ADJUSTABLE DRAWING SHELF.

(Modified from Optic Projection).

DB Drawing board with a 25 × 30 cm. glass plate in the middle for tracing on the back of photographs. It is placed on the brackets to form the adjustable shelf (*ADS*).

ls Leveling screws in the bottom of the table legs.

Rheostat The balance for regulating the electric current of the arc lamp.

c c, *ks* Electric cable and knife switch.

Table The projection table with drawer (*d*). This table is 100 cm. high, and the top 125 cm. long and 50 cm. wide. It is stained by aniline black.

ADS Adjustable shelf with a drawing board having a glass center 25 × 30 cm.

bt Bolts with thumb nuts holding the shelf at any desired height on the legs.

N R Mazda lamp and reflector to throw the light up through the picture which is being traced.

c Cable with separable cap to attach to the lighting system.

Arc Lamp The right-angled carbon arc lamp for supplying light to the projection microscope.

Condenser The three lens condenser and water bath (fig. 178).

Microscope The compound microscope with substage condenser and ocular.

m 45° mirror or prism for reflecting the light directly downward upon the drawing shelf.

Axis, *Axis* The principal optic axis.

of the light source from the large condenser falls directly on the specimen. Focus and arrange the drawing surface to give the right size and magnification, then trace the outlines and the details. Later, ink in, using the specimen to check up with. (For erect images see §§ 430–437.)

After one has had sufficient practice, the drawing can be partly or wholly completed under the projection apparatus. For this, one must light the drawing surface enough either by means of a portable lamp or by some means of letting in daylight. At the same time there must be a screen to cut off the image where one is doing the finishing. By removing the screen the image appears at any time and serves to check the work.

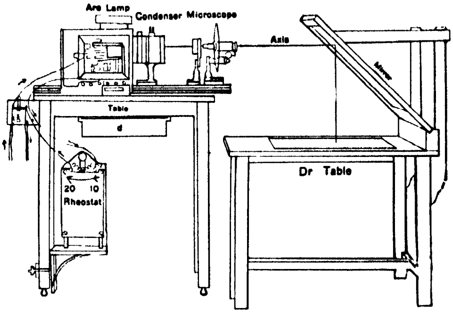


FIG. 181. PROJECTION MICROSCOPE WITH MOVABLE DRAWING TABLE AND 45° MIRROR.

(From Optic Projection).

The projection table has the dimensions given in fig. 180.

The arc lamp is automatic and the rheostat for current may be adjusted to give from 10 to 20 amperes.

The condenser is of the three lens water cell type, and the microscope with separate stage; the microscope has an amplifier in place.

The drawing table (Dr. Table) is of a convenient height for sitting beside. It is 76 cm. high and the top 100 cm. long and 75 cm. wide.

The 45° plate glass mirror is large (75 cm. long and 60 cm. wide).

§ 423. Drawing with high powers, 8 (20x) to 2 mm. (90x) focus. — For this high power drawing one should use an ocular as well as an objective, and a substage condenser in addition to the condenser of the lantern or small lamp (fig. 182), or light of sufficient aperture will not be supplied to the microscope. In using the highest powers it is also well to connect the substage condenser to the slide by homogeneous liquid, as described in § 124. The large or small arc light or the concentrated or ribbon filament, 108-watt, 6-volt headlight lamp is needed for good results.

If one has a drawing room, a large or small arc lamp, and direct current, the arrangements shown in fig. 180 are best, but if direct current is not available, excellent results can be obtained by using the small arc lamp or the 108-watt lamp on the alternating current, house electric lighting system, and the microscope, shown in figs. 182–183.

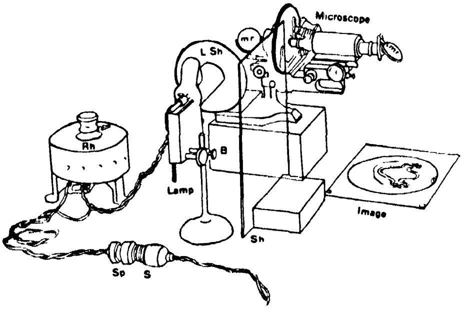


Fig. 182. Drawing Microscope with Small Arc Lamp on the House Lighting System.

(From Optic Projection).

S, Sp The lamp socket and separable attachment plug.
Rh The rheostat not allowing over 5 amperes of current to flow.
Lamp The small arc lamp at right angles to the microscope.
Microscope The microscope on a block (*B*).
mr, mr The mirror of the microscope, and the mirror over the ocular to reflect the light directly downward.
Image The picture of the microscopic object reflected down upon the drawing paper.
Sh Opaque shield to screen the light from the drawing surface.

The light supplied to the substage condenser should be approximately parallel. This is attained with the small lamp by putting the arc at the focus of the condenser. With the large lamp one should use a long focus lens for the condenser, as shown in fig. 184.

In all cases the substage condenser should be shifted up and down slightly until the best effect is produced. The substage condenser should, of course, be centered carefully before commencing to draw (§ 118).

§ **424. Drawings for publication.** — The inexpensive photographic processes of making cuts for the printing press bring within the reach of every writer the possibility of appealing to the eye by means of pictures and diagrams illustrating the facts which are presented in the text. Artistic ability is, of course, indispensable for a perfect representation, but any one willing to give the time and the pains can make simple drawings, especially if one or more of the helps above described are available.

The various helps for making drawings described in this chapter will be found useful to the born artist as well as to the person who has not great artistic ability, for by means of the optical and mechanical helps the outlines and proportions can be secured with fidelity by any one. Then the born artist can use the time saved for making the pictures more artistic, and the plodder can feel confident that his efforts are correct.

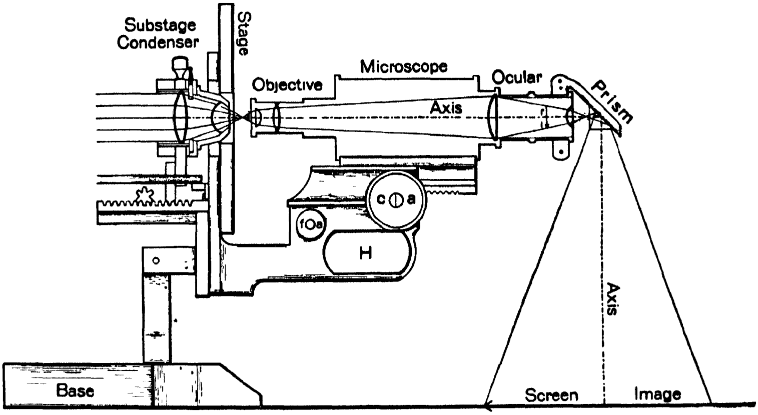


FIG. 183. THE MICROSCOPE ARRANGED FOR DRAWING ON A HORIZONTAL SURFACE.
(From Optic Projection).

The microscope is of the handle type (*H*) with the fine adjustment (*f a*) on the side below the coarse adjustment (*c a*).

The ocular is of the Huygenian form with the real image at (*r i*).

Prism, the right-angled prism beyond the ocular to reflect the light directly downward.

Young authors are urged to get the Style Brief furnished by the Wistar Institute of Philadephia. This is a guide for the preparation of manuscript and drawings for publication in the scientific journals published by the Institute. The hints to contributors given on the second page of the cover in all the journals give in a nutshell the main points. These journals are: The American Journal of Anatomy; The Anatomical Record; The Journal of Morphology; The Journal of Comparative Neurology, and The Journal of Experimental Zoölogy. The little book: Preparation of Scientific and

Technical Papers by Trelease and Yule, 2d. ed. 1927, gives excellent advice which is illustrated by abundant examples.

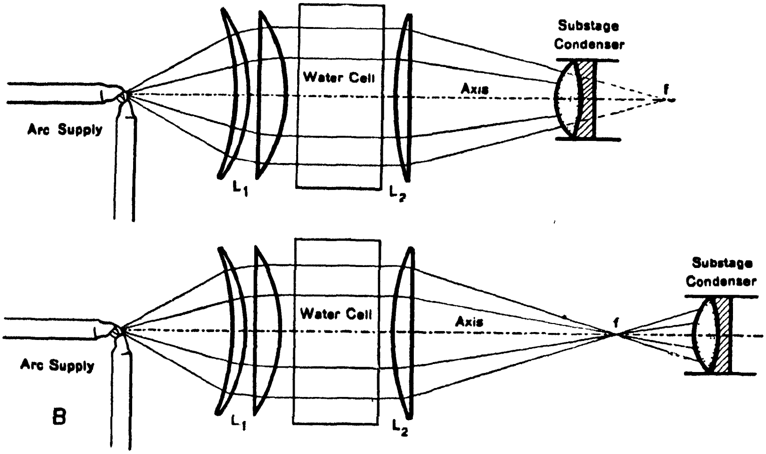


FIG. 184. DIAGRAMS TO SHOW THE POSITION OF THE SUBSTAGE CONDENSER WHEN NO PARALLELIZING LENS IS USED.

(From Optic Projection).

A The substage condenser is within the focus (*f*) at a point where the long light cone is of about the same diameter as the substage condenser.

B The substage condenser is beyond the focus (*f*) of the long focus main condenser at a point where the diverging cone is of about the same diameter as the substage condenser. This is the better position for the substage condenser of the ordinary microscope.

Arc Supply The right-angled carbons of the arc lamp.

L_1 L_2 The first and the second elements of the main condenser.

Water Cell. This is to remove the radiant heat.

Axis The principal axis on which all the parts are centered.

f The principal focus of the second element of the main condenser. In both cases the focus is long.

Substage Condenser This is the first or lowest element of the substage condenser. It is of the achromatic type.

A great many good hints can be found by studying the illustrations in well-printed books and in scientific journals, especially those dealing with the subject in which one is interested.

§ **425. Size of drawings.** — For most draughtsmen it is wise to make the drawings two or three times the size of the final cut for publication. It is easier to make the details clear, and then little defects are minimized by the reduction. The photo-engraver can make the cut any desired reduction, but one should remember that the lines should be heavy enough for the reduction desired, otherwise the finest details are likely to be lost.

§ **426. Reduction.** — There is some confusion as to the meaning of reduction in the minds of authors. For the engraver this term has a perfectly definite significance. It is linear measure, and never area or solid measure, that he considers. For example, if the engraver is directed to make the cut half the size of the drawing, he will make every line half the length of the corresponding line in the drawing. The area will then be one-fourth that of the drawing. If the cut is to be reduced to one-fourth the drawing, each line will be only one-fourth the length of the original, and the area will be one-sixteenth that of the drawing (figs. 185–186).

§ **427. Lettering drawings.** — After the drawings are finished the details must be indicated in some way. This may be by having the full name of the part, an easily intelligible abbreviation, or a letter or a numeral upon or near it (fig. 188).

The lettering should be done with discrimination in two ways:

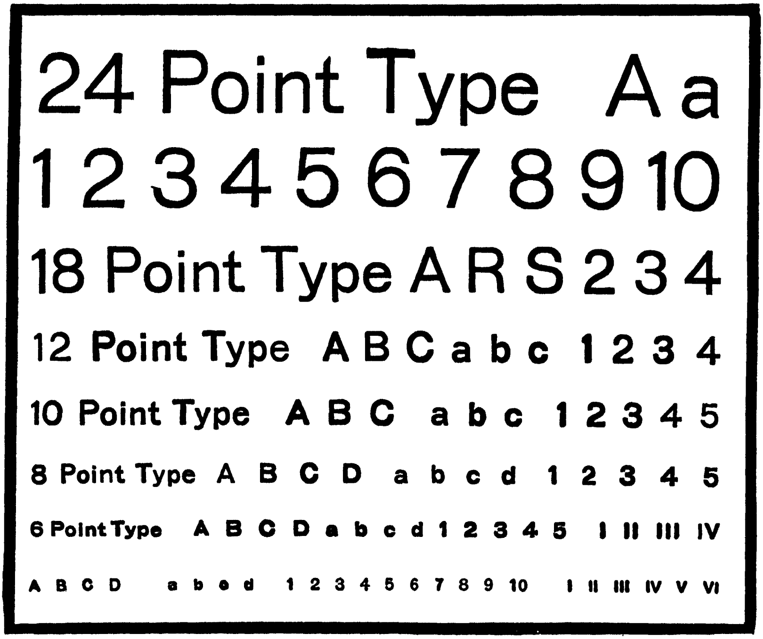
(1) The letters, words, etc., should be artistically arranged and then put on straight. For this one may need to use a T-square and straight edge. Most persons cannot letter neatly enough to letter with a pen. Printed words and letters can be pasted upon the drawing. In the final cut the appearance is as if words, letters, or numerals were printed on the picture (fig. 26.)

If the letters, abbreviations, etc., are not upon the parts they are meant to indicate, then "leaders," that is, full or broken lines should be drawn from the part to its designating letter, numeral, abbreviation, or word (figs. 18, 26).

(2) The size of type to be used should correspond to the size of the picture and the amount of reduction. The letters should not be the most prominent thing about a picture, neither should they be so small that one needs a microscope to read them. By consulting figs. 185–186 one can get a clear notion of the appearance of various sizes of letters when reduced. If one has a camera (fig. 174), it is a good plan to put letters of different sizes upon the drawing and

then, having the bellows set to give the reduction desired, look at the image of the drawing and lettering and see how they will look in the final picture.

For photo-engraving, Gothic type gives the best results (fig. 185).



24 Point Type A a

1 2 3 4 5 6 7 8 9 10

18 Point Type A R S 2 3 4

12 Point Type A B C a b c 1 2 3 4

10 Point Type A B C a b c 1 2 3 4 5

8 Point Type A B C D a b c d 1 2 3 4 5

6 Point Type A B C D a b c d 1 2 3 4 5 I II III IV

A B C D a b c d 1 2 3 4 5 6 7 8 9 10 I II III IV V VI

FIG. 185. GOTHIC TYPE FOR LETTERING DRAWINGS.
(From Optic Projection).

§ 428. **Fastening the letters to the drawing.** — The letters, etc., should be printed on thin, smooth, very white paper, and they should be black, not gray. Tissue paper is often used, but that is not so easy to handle as a paper about like the so-called "Bible paper."

The words, letters, and numerals for a drawing are cut out and arranged on the drawing to get the best effect. Then using a T-square and straight edge, each letter or word is stuck to the drawing in the proper position as follows: Some fresh starch paste is made by placing in a small tin or aluminum dish 5 grams of laundry starch and adding 50 cc. of cold water. Stir with a spoon and then heat gradually with constant stirring on a stove or over a gas flame

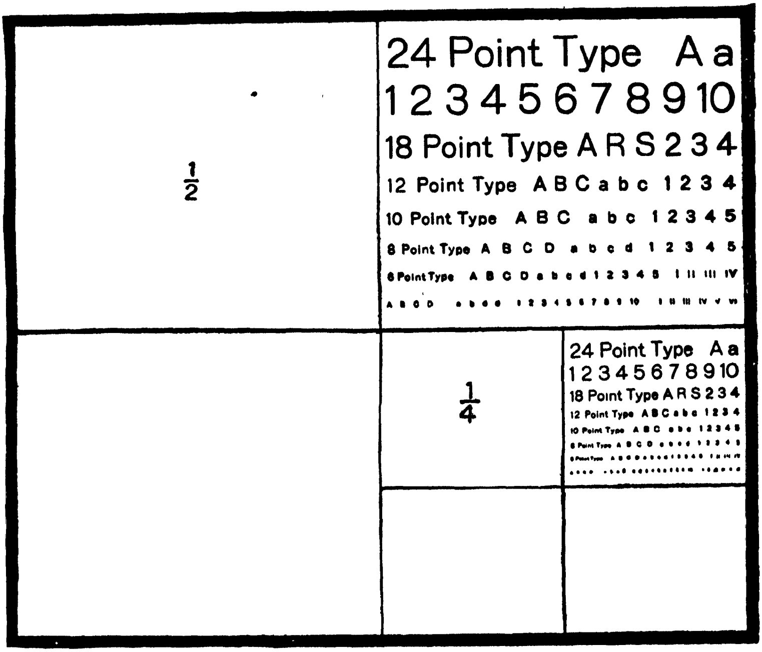

1/2
24 Point Type Aa
12345678910
18 Point Type A R S 2 3 4
12 Point Type A B C a b c 1 2 3 4
10 Point Type A B C a b c 1 2 3 4 5
8 Point Type A B C D a b c d 1 2 3 4 5
6 Point Type A B C D a b c d 1 2 3 4 5 I II III IV
1/4
24 Point Type Aa
12345678910
18 Point Type A R S 2 3 4
12 Point Type A B C a b c 1 2 3 4

FIG. 186. THE GOTHIC TYPE IN FIG. 185 REDUCED TO ONE-HALF AND TO ONE-FOURTH NATURAL SIZE.

(From Optic Projection).

until the paste is formed. Mucilage and paste which has been made for some time are not good for pasting the letters. Mucilage turns the paper yellow and the old paste is lumpy. Any good library paste will answer, also stainless rubber cement.

Use a fine brush to put the paste on the letters, and then use fine forceps (fig. 138) to pick up the letters and transfer them to the proper position. Press down with the finger covered with tissue paper or very fine cloth or with fine blotting paper. Press directly downward or the letter is likely to be displaced or distorted by a lateral thrust.

§ **429. White letters for black background.** — The white letters, words, or numerals are most easily procured by photography. The letters, words, etc., are printed on tissue paper. This is used as a negative by placing it face down on a glass plate and in a printing frame. Use some developing paper, of the contrast variety. Print as for any negative and develop with a contrast developer so that the whites and blacks will be perfect. The white letters, etc., are then cut out and pasted on the drawing as described above. This photographic paper is rather thick and will show a white edge where it is cut. Blacken the white edges of the letters or words with carbon ink after the letters are stuck in place (fig. 188).

AVOIDANCE OF INVERSION

§ **430.** It is desirable to make drawings like the object without any inversion whatsoever, provided the object has rights and lefts, etc. For structural detail like cells, etc., it makes no difference whether the image is erect or not, but with symmetrical organs and animals it is very confusing to have the parts inverted in the drawing. For example, it is unsatisfactory to have the liver shown as if on the left side and the heart on the right side.

In order to avoid inversions, it is necessary to know what inversions are produced by the different optical appliances used to assist in drawing. Then one can so arrange the object that the image will be exactly like the object. It is believed that the following directions will enable the worker to arrange his specimen and the apparatus so that erect images may be produced without undue effort.

The simplest of all ways to get the image without inversion is to arrange the slide on a piece of white paper so that the object is erect and then to write with a very fine pen the letters a, k, on the cover-glass of the specimen to be drawn (fig. 187). Now with the low power (16 to 60 mm. [10x to 2.66x]) objective, project the image of the specimen and letters upon the drawing paper. One can then continue to rearrange the slide until the letters are erect; the specimen will then also be erect.

§ **431. Images to be traced in the photographic camera.** — These images are wrong side up and the rights and lefts are reversed. This can be corrected by drawing the picture on the tracing paper in the inverted position and then inverting the tracing after it is finished; or the specimen can be put in the inverted position, then the image will be erect.

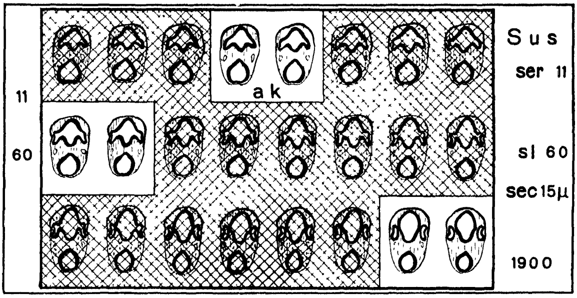


FIG. 187. SLIDE OF SERIAL SECTIONS, SHOWING THE DEVELOPMENT OF THE EYE WITH THE LETTERS a k, TO AID IN GETTING ERECT IMAGES IN DRAWING WITH PROJECTION APPARATUS.

(From Optic Projection).

This slide is also to show how to mask preparations which are to be used in class demonstration.

Demonstrate this by putting the metric card in position and tracing some of the larger letters or figures on the tracing paper. Then turn the drawing paper around 180° and the letters or figures will appear erect.

Put the metric card wrong edge up to start with; then the letters or figures will appear right side up on the tracing paper.

§ **432. The use of a negative for projection and tracing.** — Put the face of the negative that reads correctly next the source of light and wrong edge up; then it will appear erect in every way on the drawing paper. This is the way lantern slides are put in the holder.

§ **433. The Wollaston or Abbe camera lucida.** — With these camera lucidas there are two reflections of the rays (figs. 168–169), consequently there is no inversion produced by the camera, but the microscope inverts the image the same as the photographic objective, and erect images are obtained either by inverting the drawing after it is made or by putting the object in an inverted position under the microscope, just as with the photographic camera.

Demonstrate that this will produce erect drawings by using the letters (fig. 187) and making sketches of their images by the camera lucida, having the letters right edge up on the stage in one case and wrong edge up in one.

ERECT IMAGES WITH THE PROJECTION MICROSCOPE

§ **434. Erect images with an objective only or with an objective and amplifier.** — There are two cases: (1) When opaque drawing paper is used. In this case the object must be put on the stage with the cover-glass toward the light and the slide toward the objective, and it must be lower edge up. Only low powers (16 mm. (10x) and lower objectives) should be used, for the thick slide introduces aberrations (fig. 64) and is likely to be too thick for the free working distance (fig. 52 *B*).

(2) When a translucent drawing paper is used and the drawing is made on the back. In this case the specimen is put on the stage lower edge up, but with the cover-glass facing the objective. All powers can be used. This is similar to the conditions described for the photographic camera where the tracing paper is used on the clear glass (§ 431).

Test the correctness of the directions by using a preparation with the letters a, k, on the cover-glass (§ 430, fig. 187).

§ **435. Erect images with an objective or an objective and an amplifier and a prism or 45° mirror.** — Place the specimen on the stage lower edge up and with the cover-glass toward the objective. The image will be erect on the opaque drawing paper. Test with the lettered specimen (fig. 187).

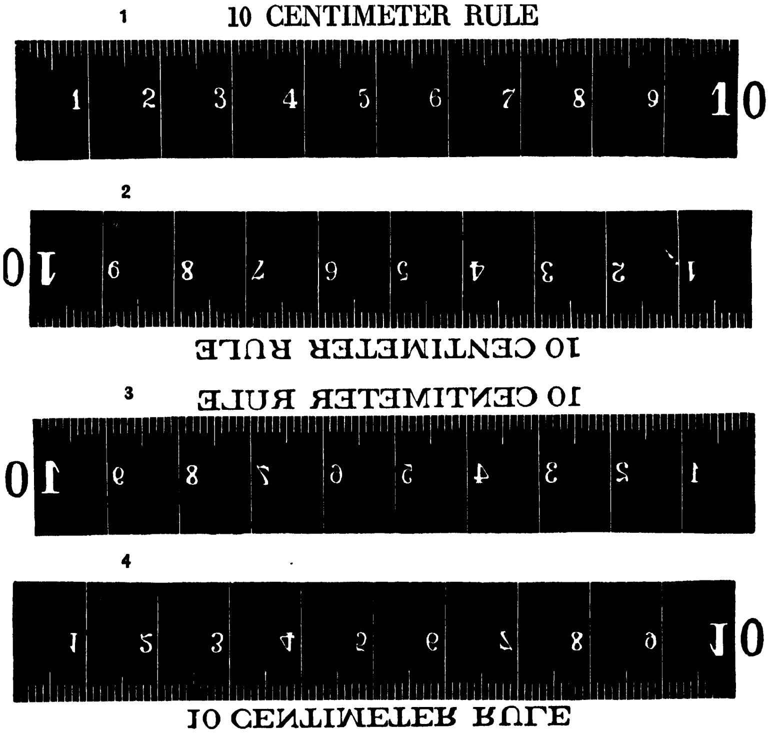


Fig. 188. 1, 2, 3, 4, Erect and Inverted Images of the Metric Scale. (From Optic Projection).

1. Erect image. 2. Inverted image. 3. Mirror image. 4. Inverted mirror image.

§ **436. Erect images with an objective and an ocular.** —

(1) Opaque drawing paper. Place the specimen on the stage right edge up, but with the cover-glass facing the light, the slide toward the objective.

(2) Translucent drawing paper. If the drawing can be made on the back of translucent paper the specimen is placed on the stage right edge up and with the cover-glass facing the objective. Test with the lettered specimen (fig. 187).

§ **437. Erect images with an objective and ocular and a 45°**

mirror or prism. — Place the slide on the stage top edge up, and with the cover-glass facing the objective. The image will be erect on an opaque drawing surface. Test with the lettered preparation (fig. 187).

CLASS DEMONSTRATIONS

§ **438. Demonstration microscopes.** — Ever since the microscope was invented physicians and naturalists have made the greatest use of it for demonstration purposes. It was a favorite expression of the older writers that the instrument had created a new world of the minute. Naturally in the beginning each person used the instrument for himself as with the simple microscopes of Roger Bacon. However, soon after the invention of the compound microscope Kepler and Scheiner discovered the way to get projection pictures, and these have been much used for demonstrating to groups of people the enlarged screen pictures.

Recently the powerful lime and electric lights have made it possible to carry on these demonstrations to an extent beyond the hopes of the earlier workers; and have put into the hands of the teacher facilities which are beyond estimation in value for helping students. Still for many things and for many persons having charge of large classes, the individual simple or compound microscope is still and always will be much used.

DEMONSTRATION MICROSCOPES AND INDICATORS

§ **439. Simple Microscope.** — Holding the simple microscope in one hand and the specimen in the other has always been used for demonstration, but for class demonstration it is necessary to have microscope and specimen together or the part to be observed by the class is frequently missed. Originally blocks of various kinds to hold both microscope and specimen were devised, but within the last few years excellent pieces of apparatus have been devised by several opticians for the purpose.

The tripod magnifier and various pocket magnifiers are excellent for the purpose (figs. 15–16). Where the microscope and object should be held in a fixed position, the focusing stand for the simple microscope is good (fig. 17).

§ **440. Compound demonstration microscope.** — This was originally called a clinical or pocket microscope. It is thus described by Mayall in his Cantor Lectures on the history of the microscope: "A small microscope was devised by Tolles for clinical purposes which seems to me so good in every way that I must ask special attention for it. The objective is screwed into a sliding tube, and for roughly focusing the sliding motion suffices; for fine adjustment, the sheath is made to turn on a fine screw thread on a cylindrical tube, which serves also as a socket carrier for the stage. The compound microscope is here reduced to the simplest form I have met with to be a really serviceable instrument for the purpose in view; and the mechanism is of thoroughly substantial character. I commend this model to the notice of our opticians."

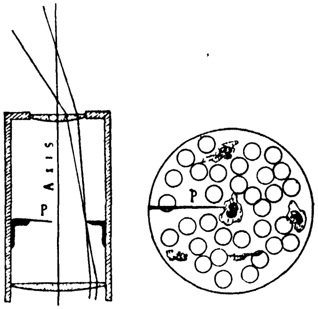


FIG. 189 A, B. POINTER OCULAR AND FIELD WITH POINTER.

A. POINTER OR INDICATOR OCULAR WITH A CAMEL'S HAIR (*P*) STUCK TO THE OCULAR DIAPHRAGM AND EXTENDING OUT INTO THE OPEN SPACE WHERE THE REAL IMAGE IS FORMED.

B. THE MICROSCOPIC FIELD OF A BLOOD PREPARATION WITH THE POINTER (*P*) DIRECTED TOWARD A LEUCOCYTE.

Since its introduction by Tolles many opticians have produced excellent demonstration microscopes of this type, but most of them have not preserved a special mechanism for fine adjustment. With it one can demonstrate with an objective of 6 mm. (30x) satisfactorily. It has a lock, so that once the specimen is in the right position and the instrument focused it may be passed around the class. For observation it is necessary for each student only to point the microscope toward a window or a lamp.

A modification of this clinical microscope was made by Zentmayer, in which the microscope was mounted on a board, and a lamp for illuminating the object was placed at the right position.

§ **441. Traveling Microscope.** — Many years ago the French opticians produced most excellent traveling microscopes. Now the opticians of America and other countries make serviceable instruments. For the needs of the pathologist and sanitary inspector a microscope must possess compactness and also the qualities which render it usable for nearly all the purposes required in a laboratory. This instrument is a type of much apparatus which has grown up with the needs of advancing knowledge.

§ **442. Indicator or pointer ocular.** — This is an ocular in which a delicate pointer of some kind is placed at the level where the real image of the microscope is produced. It is placed at the same level as the ocular micrometer, and the pointer, like the micrometer, is magnified with the real image and appears as a part of the projected image (fig. 189 *B*). By rotating the ocular or the pointer any part of the real image may be pointed out as one uses a pointer on a wall or blackboard diagram. By means of the indicator eyepiece one can be certain that the student sees the desired object, and is not confused by the multitude of other things present in the field. This device has been invented many times. It illustrates well the adage: "Necessity is the mother of invention," for what teacher has not been in despair many times when trying to make a student see a definite object and neglect the numerous other objects in the field? So far as the writer has been able to learn, Quekett was the first to introduce an indicator ocular with a metal pointer which was ad-

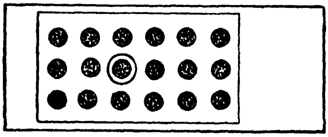


FIG. 190. RING AROUND ONE OF THE SECTIONS OF A SERIES FOR DEMONSTRATING SOME ORGAN ESPECIALLY WELL.

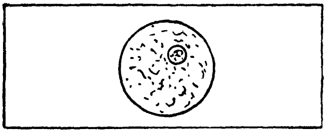


FIG. 191. A MICROSCOPIC PREPARATION WITH A RING AROUND A SMALL PART TO SHOW THE POSITION OF SOME STRUCTURAL FEATURE.

justable and could be turned to any part of the field or wholly out of the field.

It is not known who adopted the simple device of putting a fine hair on the diaphragm of the ocular, as shown in fig. 189. This may be done with any ocular, positive or negative. One may use a little mucilage, Canada balsam, or any other cement to stick the hair on the upper face of the diaphragm so that it projects about halfway across the opening. When the eye-lens of the Huygenian ocular is screwed back in place, the hair should be in focus. If it is not, screw the eye-lens out a little and look again. If it is not now sharp, the hair is a little too high and should be depressed a little. If it is less distinct on screwing out the ocular, it is too low and should be elevated. One can soon get it in exact focus. Of course it may be removed at any time. Ordinary hair is too coarse. The tip of one of the hairs in a camel's hair brush is excellent.

THE PROJECTION MICROSCOPE

§ **443. Projection Microscope.** — One of the most useful and satisfactory means at the disposal of the teacher of microscopic anatomy and embryology for class demonstrations is the projection microscope. With it he can show hundreds of students as well as one, the objects which come within the range of the instrument.

It is far more satisfactory than microscopic demonstrations, for with the projection microscope the teacher can point out on the screen the structural features and organs which he wishes to demonstrate, and he can thus be certain that the students know exactly what is to be studied. Unless one employs a pointer ocular (fig. 189), there is no certainty that the student selects from the multitude of things in the microscopic field the one which is meant by the teacher. Like all other means, however, the projection microscope is limited. With it one can show organs both adult and embryonic, and the general morphology. For the accurate demonstration of cells and cell structure the microscope itself must be used. As a general statement concerning the use of the projection microscope for demonstration purposes, it may be said that it is entirely satisfactory for objects and details which show under the microscope with objectives up to 16 mm. (10x) equivalent focus. For objects and details requiring objectives higher than 16 mm. (10x) focus in ordinary microscopic observations, the projection microscope is unsatisfactory with large classes.

With small classes (10 or 15) where the screen distance can be reduced to about one meter, demonstrations with oil immersion objectives are satisfactory. However, when the finest details of structure are to be seen most successfully under high powers, each individual must look into a microscope for himself and attend to all the finer adjustment and lighting.

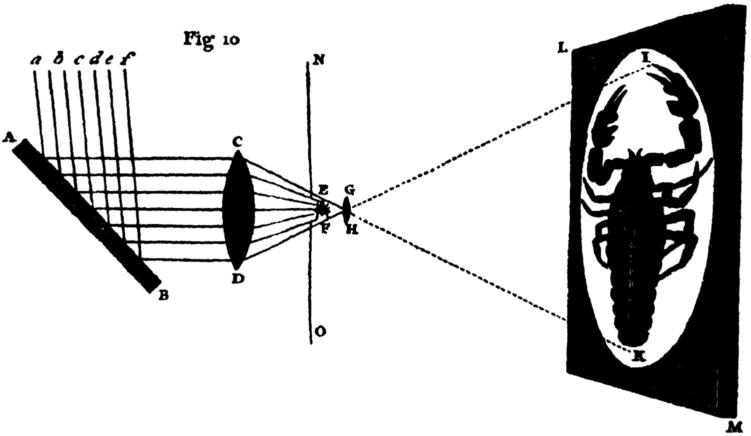


FIG. 192. DIAGRAM OF ADAMS' SOLAR MICROSCOPE. THIS ILLUSTRATES WELL THE ADVANTAGE OF SOME FORM OF PROJECTION MICROSCOPE FOR DEMONSTRATION PURPOSES.

§ **444. Euscope for testing laboratories and for demonstration.** — In 1924, Dr. W. G. Exton described in the Jour. Amer. Med. Assoc., Vol. 82, pp. 1838–1840, a device to enable the observers in testing laboratories to look at the microscopic image with both eyes. It is a small self-contained projection microscope. The microscope is vertical and over it is fitted a pyramidal box with a screen at the far end to receive and reflect the image. At the top of the tube receiving the tube of the microscope is a totally reflecting prism which projects the originally vertical beam horizontally to the screen. For demonstrations to a small group the opaque screen is removed and an extension put in its place. At the far end of the pyramidal extension is a screen of finely ground glass. The illumination is by a small arc light or by one of the 108-watt, 6-volt lamps (figs. 78–80).

For individual use, the observer looks into a kind of hood which makes it possible to use the instrument in a light room. Although not an essential part of the apparatus, there is a special magnifying lens at the eye end of the instrument into which the observer looks. This instrument in the hands of the author has served an excellent purpose for demonstration to small groups. It is more satisfactory with low than with high powers. The Euscope is manufactured by the Bausch & Lomb Optical Co., and is fully described and illustrated in their catalogue, pp. 304–305.

CONDUCT OF A DEMONSTRATION WITH THE PROJECTION MICROSCOPE

§ **445. Preparedness.** — From the great difficulty in making really good projection demonstrations with the microscope the preparation should be thorough. The following are some of the most important things to look after:

(1) If any of the objectives used are of the photographic type and have an iris diaphragm, that should be opened to the fullest possible extent.

(2) The microscopic slides to be used should be in order so that they can be grasped easily.

(3) If the slides have many sections upon them, as in a series, then the slide should be masked by putting some orange paper over the cover-glass with openings for the sections to be shown; then these can be found quickly and with certainty (fig. 193).

(4) Indicate in some way which edge of the slide should be up. This will save time, and add to the respect for the exhibition.

(5) It is often a great help to have stated on the preparation the objectives best adapted to bring out the special feature desired.

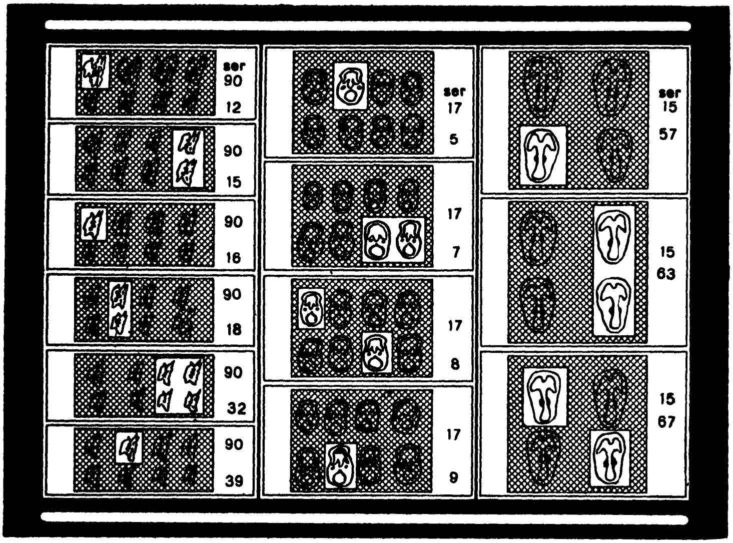


FIG. 193. SLIDE TRAY WITH MASKED PREPARATIONS TO BE USED IN A DEMONSTRATION.

(From Optic Projection).

(6) For holding the specimens, a slide tray may be used (fig. 193) or one of the slide boxes. In any case they must be so that the slides can be grasped easily.

(7) It is for many lecturers easier to manipulate the projection microscope themselves and to use a pointer held out in the cone of light. The pointer appears as sharply as when put on the screen.

(8) For all but the highest powers a substage condenser is not needed; and one can light objects up to 50 or 60 mm. in diameter if the object is placed in the right position in the cone of light (fig. 194).

(9) For objectives of higher power than 4 mm. (40x) a substage condenser should be used, and if an ocular is used as well as an ob-

jective then the substage condenser is advantageous for powers above 8 mm. equivalent focus. For lighting see §§ 119, 122, 477.

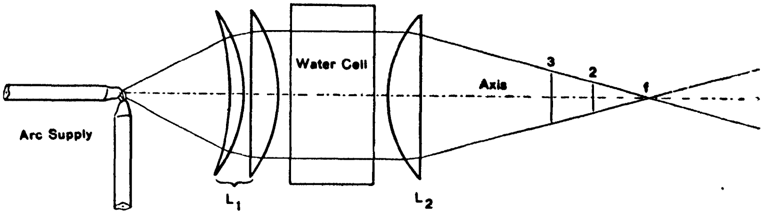


FIG. 194. ILLUMINATING OBJECTS OF VARIOUS SIZES IN MICRO-PROJECTION WITH THE MAIN CONDENSER ONLY.

(From Optic Projection).

The object must be put in the cone of light at a point where it will be fully illuminated.

For high powers it will be at or very near the focus (*f*). For larger objects and low powers at 2 or 3, or even closer to the condenser face.

Arc Supply The right-angled carbons of the arc lamp.

L_1 L_2 The first and second elements of the triple condenser.

Water Cell The water cell for absorbing radiant heat (§ 446). It is in the parallel beam between the first and second elements of the condenser.

Axis The principal optic axis on which all the parts are centered.

(10) One of the most important points is to have a very white screen. A cloth or wall screen painted with artist's scenic white gives a very perfect screen which does not yellow with age, and it is restored by an occasional coat of fresh white. Semi-mirror screens are successful only in narrow rooms.

For short screen distances (1 or 2 meter screen distances) white cardboard or a sheet of very white bristol board gives excellent results.

The apparatus, in contrast to the screen, should be dull black.

§ 446. Heat-absorbing glass and water cell; objectives, amplifiers and oculars. — As the cone of light from the condenser (fig. 194) must be focused on the object, the object is likely to be overheated and spoiled by the longer waves of radiant energy in the light or accompanying it. These longer waves represent about 90 per cent of the radiant energy from an artificial light source like the arc lamp, and the visible waves only about 10 per cent.

If in some way the invisible 90 per cent of energy could be absorbed, a larger amount of energy represented by light could be used and the pictures made more brilliant without injuring delicate specimens or living objects. This possibility has been realized by the combination of heat-absorbing glass and a water cell by Dr. H. P. Gage of the Corning Glass Works. (See Trans. Soc. Mov. Pict. Eng. May, 1924, pp. 38, 42).

With this combination, osmic acid preparation, Golgi and Weigert stained sections and living infusoria have been projected from half

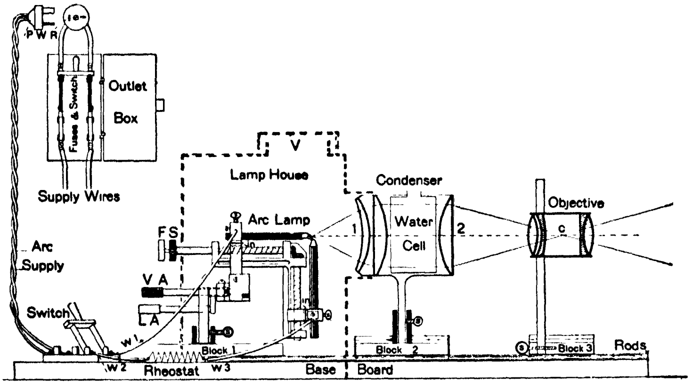


FIG. 195. PROJECTION APPARATUS SHOWING THE PARTS AND THE WIRING FOR AN ARC LAMP.

(From Optic Projection).

The Objective, *Condenser*, and *Arc Lamp* are on separate blocks which move independently along the optical bench.

c Center of the objectives where the rays from the condenser should cross.

1, *2* The first and second elements of the three lens condenser with a water cell for absorbing radiant heat between the lenses (§446).

V The ventilating hood of the lamp house.

LA, *VA* The mechanism for fine adjustment of the arc lamp to the sides and vertically. These are a necessity for projecting with the microscope, otherwise the crater cannot be kept centered.

FS The fine adjustments for the two carbons.

PWR Separable attachment for the wires from the outlet box to the table switch.

W_1 ·Wire from the table switch to the upper carbon.

W_2 Wire from the table switch to the rheostat.

W_3 Wire from the rheostat to the lower carbon.

a minute to 10 minutes and longer without injury when a 10 ampere, direct current was used for the arc light.

For individual use, objectives and oculars of all powers can be employed for projection. For classes it is not satisfactory to use objectives higher than 8 mm. (20x) to 4 mm. (40x); and with the larger classes it is better to use the large condenser (fig. 194) without a substage condenser, and to use the objective or an objective and amplifier for projection. A much larger and more brilliant field can be shown in this way than when an ocular is used, especially when the narrow tube for the ocular is removed and a wide (5 cm.) tube is present on the projection outfit.

§ **447. Centering the optical parts on one axis.** — This is one of the most important procedures of all and no good projection can be accomplished without it. The easiest way is first to arrange the crater of the arc lamp, the central point of the large condenser, and the microscope objective all at the same height from the baseboard (fig. 179). If then the lamp is turned on and the objective placed in the focus of the main condenser cone, the image of the crater of the arc lamp should be formed on the end of the objective, the brightest part on the front lens. If the image is to one side, above or below, then the microscope should be raised, or lowered. After being once carefully centered, the centering will vary slightly with the burning of the carbons. To compensate for this there must be fine adjustments to raise and lower the carbons and to move them from side to side. No good projection can take place unless the full cone of light shines upon the end of the objective.

To get the very best effect in the easiest way, there should be a dull black shield over the end of the objective (fig. 196) so that the image of the crater can be seen without hurting the eyes. When the crater is focused on the end of the objective, the specimen is moved up until it is in focus, the objective not being moved. Of course this means that the stage must be separately movable (fig. 179). See also § 422.

§ **448. Demonstrations with a vertical projection microscope.** — Many specimens must be mounted in liquids and cannot be set in a vertical position; therefore the microscope must be vertical and the object remain horizontal. In such a case project the light from the large condenser (fig. 179) or from the small arc lamp (fig. 78) upon the mirror of the microscope and reflect it directly upward, and then use a mirror or prism to change the direction from vertical to horizontal. (See figs. 180, 183, to recall how the beam is changed in direction 90°).

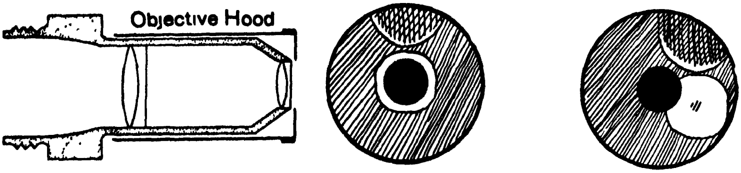


FIG. 196. METAL HOOD OVER THE OBJECTIVE TO AID IN CENTERING THE LIGHT.

(From Optic Projection).

A Longitudinal section of the objective to show the metal hood.
B End view of the objective with the crater of the arc lamp directly in the center, at the left, and to one side of the center at the right. The adjustments, *VA*, *LA* in fig. 195 are to enable one to center the light easily.

A most striking preparation is one of the hay infusion (§ 350) projected upon the screen. A water immersion objective of 2 to 3 mm. equivalent focus is excellent for projecting such preparations. It is especially necessary to have a sheet of heat-absorbing glass somewhere in the light beam before it reaches the living organisms (§ 446).

Demonstration Lantern and Table for Artificial Daylight

§ **449. Special microscopic demonstrations.** — As stated above, if one is to see the finest details of structure, there is no satisfactory way but to look into the microscope direct. There is also in every laboratory for microscopic work considerable waste space if dependence is put upon daylight. If artificial light is used regularly, the method here given is also applicable.

The main points for this kind of demonstration were worked out by Dr. B. F. Kingsbury for his laboratory of histology and embryology.

A round top demonstration table of a size for 8 microscopes is made and in the middle a single mazda lamp of 200 or 250 watts is installed (fig. 197). Around this lamp are 8 shields, each containing a piece of daylight glass.

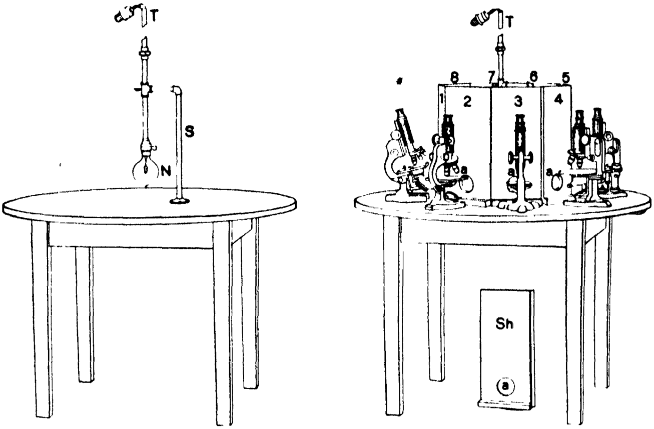


FIG. 197. KINGSBURY'S DEMONSTRATION TABLE WITH ARTIFICIAL DAYLIGHT. (ABOUT $\frac{1}{25}$ NATURAL SIZE).

(From the Anatomical Record, June, 1916).

T Top of the metal tube and the separable attachment plug. This tube reaches about 2 meters above the floor so that the supply cable will be out of the way.

N S The single 250-watt mazda lamp with its metal support.

1, 2, 3, 4, 5, 6, 7, 8 The shields (*SH*) with a disc of daylight glass (a) in each at the level of the microscope mirror.

With this arrangement 8 microscopes can be used at once (fig. 197) and the light is sufficient to enable the student to use all powers of the microscope up to the highest oil immersion. This method of demonstration has already been in use during the college years of 1915–1931 and has proved successful beyond expectation.

§ 450. Demonstrations with the polarizing and the ultra-violet microscopes. — For the best effects with polarized light a dark or at least a dimly lighted room is most successful. That is, no light should get to the eye that has not passed through the microscope.

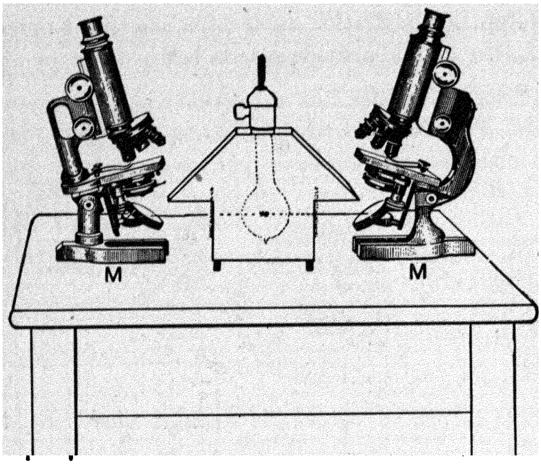


FIG. 198. TWO MICROSCOPES AND A CHALET LAMP ON A LABORATORY TABLE.
(Above one-ninth natural size).

The Chalet microscope lamp with two windows (fig. 46) serves well for two observers on opposite side of the same table, or two tables may be placed side by side and the lamp rested partly on each.

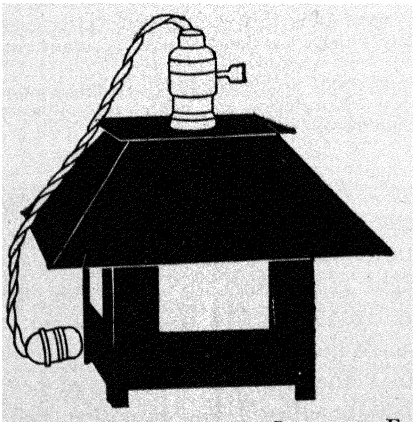


FIG. 199. CHALET MICROSCOPE DAYLIGHT LAMP WITH FOUR WINDOWS.
(From the catalogue of the Spencer Lens Co.).

This lamp serves well for demonstrations with four microscopes. It is also good for use by four students if on a square-top table.

For the ultra-violet microscope the fluorescent light is relatively so faint that it is almost necessary to work in a darkened room. The eyes are then adjusted to twilight vision, and the delicate radiance may be seen with much satisfaction. See also the discussions in Chapters V and VI.

COLLATERAL READING FOR CHAPTER IX

ATWELL, W. J. — On the conversion of a photograph into a line drawing. Anat. Record, Vol. 10, pp. 39–41. The lines are made on the face of the photograph, then the photographic image is bleached out by means of hypo and cyanide.
COMSTOCK, J. H. — The Wings of Insects, 1918. Blue prints were made and the India ink lines made on their face. The blue was then bleached with potassium oxalate, etc.
GAGE, S. H. AND H. P., Optic Projection, Ch. X.
HARDESTY AND LEE. Laboratory drawing.
TRELEASE, S. AND YULE, E. S. — Preparation of Scientific and Technical Papers, 2d ed., 1927.

CHAPTER X

PHOTOGRAPHING EMBRYOS AND SMALL ANIMALS; PHOTOGRAPHIC ENLARGEMENTS; PHOTOGRAPHING WITH THE MICROSCOPE

§§ 451-507; FIGURES 200-214

PHOTOGRAPHY

§ **451.** From the beginning of the art of photography scientific men have used it to paint for them the forms in nature and the complex structures found in the physical and the biological world; and it has been so good a servant that it is more and more called into requisition to delineate all the phenomena as well as the forms of nature and art. This is especially true now that successful methods of color photography have become available.

§ **452. Photography with a horizontal camera.** — The most convenient position for the camera obscura is the horizontal one, and for most of the photography actually done it is very easy to arrange the objects to be photographed in a vertical position, but for much of the photography of science it is very convenient to use a vertical camera, leaving the objects in a horizontal position. With objects in liquids this is a practical necessity.

§ **453. Photography with a vertical camera.** — The object can be left horizontal as well as the camera by the use of a mirror or totally reflecting prism, but this gives the inversion of a plane mirror, and as shown in § 435 it will render the image erect on the film side of the negative, but when the negative is printed the image will be inverted. To meet all the difficulties the object may be left in a horizontal position and the camera made vertical (fig. 200).

Since 1879 such a camera has been in use in the Anatomical Department of Cornell University for photographing all kinds of specimens; among these, fresh brains and hardened brains have been photographed without the slightest injury to them. Furthermore, as many specimens are so delicate that they will not support their own weight, they may be photographed under alcohol or water with a vertical camera and the result will be satisfactory as a photograph and harmless to the specimen.

A great field is also open for obtaining lifelike portraits of water animals. Chloretoned or etherized animals are put into a vessel of water with a contrasting background and arranged as desired, then photographed. Fins have something of their natural appearance and gills of branchiate salamanders float out in the water in a natural way. In case the fish tends to float in the water a little mercury injected into the abdomen or intestine will serve as ballast. The photographs obtainable in water are almost if not quite as sharp as those made in air. Even the corrugations on the scales of such fishes as the sucker (*Catostomus teres*) show with great clearness.

While the use of photography diminishes the labor of artists about one-half, it increases that of the preparator; and herein lies one of its chief merits. The photographs being exact images of the preparations, the tendency will be to make them with greater care and delicacy, and the result will be less imagination and more reality in published scientific figures. The objects prepared with such care are more likely to be preserved for future reference.

In the use of photography for figures several considerations arise: (1) the avoidance of distortion; (2) the adjustment of the camera to obtain an image of the desired size; (3) focusing; (4) lighting and arranging the object.

(1) While the camera delineates rapidly, the image is liable to distortion. I believe opticians are agreed that, in order to obtain correct photographic images, the objective must be properly made, and the plane of the object must be parallel to the plane of the ground-glass. Furthermore, as most of the objects in natural history have not plane surfaces, but are situated in several planes at different levels, the whole object may be made distinct by using a long focus objective and a small diaphragm.

§ **454. Scale of photographs.** — It is desirable to make all photographs at some definite scale. To do this without much waste of time the camera should be calibrated for each objective that is to be used. This is accomplished easily by using a metric scale like that shown in fig. 173. By lengthening and shortening the bellows of the camera so that the image distance is greater and less, one can get the exact position for a group of magnifications and reductions. If the length of the bellows is noted for each size, and the distance of the objective from the object when the focus is good is also noted, one can arrange the camera very quickly for any special size which may be desired. The sizes found very useful by the author are: $\frac{1}{5}$; $\frac{1}{4}$; $\frac{1}{3}$; $\frac{1}{2}$; 1; 2; 2.5; 4; 5. For magnifications above 5 it is better to make a negative natural size and then make an enlargement of this, as explained in § 484.

The vertical camera shown in fig. 200 has the supporting rod graduated in centimeters and half centimeters. After the extension of the camera for any size has been once determined, it is easily made the same at some future time.

§ **455. Magnification rod for the camera.** — Objects vary so much in thickness that the focusing range of the camera should be considerable. With the ordinary camera there is usually no provision for moving the camera as a whole for focusing. With the vertical camera shown in fig. 200, where both ends of the camera must be clamped, it is difficult to focus over a large range and keep the length of camera needed for the desired magnification or reduction. For this reason the same device was applied to it as to the original vertical camera of 1879, viz., a rod passing from end to end of the camera, fixed at one end and clamped at the other. When the camera is extended the exact amount required for the size in a given case, the clamp is fixed so that the length of the camera cannot be changed; then the whole camera may be moved for focusing without any danger of varying the magnification. This device saves a great deal of time. In the original camera of 1879, the rod was graduated in centimeters. This, of course, helps to give the proper extension with the least outlay of trouble. In fig. 200 the vertical supporting rod is graduated in centimeters and half centimeters.

§ **456. Lighting for the vertical camera.** — The object should be so arranged that all the details come out with the greatest distinctness. As the light must be largely from the side, it is often necessary to put a piece of white blotting paper or cardboard on the side of the specimen opposite the window. Occasionally for lighting up deep cavities it is a great advantage to use a mirror and reflect sunlight or lamplight into the cavities for a part of the exposure.

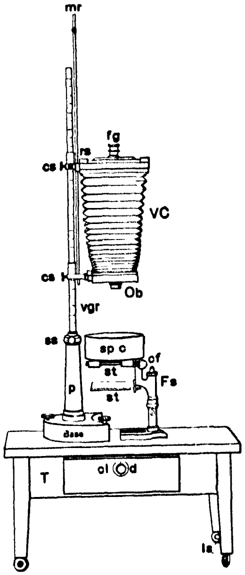


Fig. 200. Vertical Photographic Camera.

T Low table 50 cm. high, 50 cm. wide, and 70 cm. long.

Fs Focusing stand with vessel for holding embryos and small animals to be photographed under liquid.

VC Vertical camera with an objective (*ob.*) in the lower end and a focusing glass (*fg*) above. (See fig. 174 for fuller description.)

Great care must be taken in selecting a suitable background so that the specimen will stand out clearly and not be merged into the background.

When a white background is used, the shadow of the specimen is often very troublesome, and to distinguish the outline of the object W. E. Rumsey (Canadian Entomologist, 1896, p. 84) hit upon the plan of placing the object on a glass plate and putting the background on a stage below (fig. 200). A background on the lower stage does away with the confusion. If daylight is not available, excellent photographs can be obtained with mazda lamps with metallic or white reflectors to direct the light. It is usually better to employ two portable lamps and arrange them so that the shadows will not be too prominent.

§ **457. — Photographing embryos, small animals, and organs.** — The camera shown in fig. 200 is admirably adapted for this, as the objects, many of them, must be photographed under water, alcohol or other liquids.

If one has a good place to do the work in, the light can usually be arranged satisfactorily with the object in a vessel with a proper background in the bottom. If not, a double stage must be used, as shown in fig. 200.

If white embryos or other light objects are to be photographed a black background is best. This is produced by using black glass on the bottom of the dish. If black glass is not available, a good background can be produced by smooth white paper blackened with water-proof carbon ink.

With a proper background make sure that the lighting is such as to bring out the desired details. Turn the object in various positions till the desired one is found which shows clearly the points that are to be emphasized.

§ **458. Focusing stand for the vertical camera.** — To hold the specimen and to provide for the finest focusing, and also some of the coarse focusing, a modified microscope stand is convenient. It has no tube, but two stages are attached to the support usually carrying the tube. This then can be raised and lowered by the coarse and by the fine adjustment, as in focusing the microscope, except that here the stages move, the photographic objective remaining stationary (fig. 200). With the rod to hold the camera at a fixed extension, most of the focusing can be accomplished by sliding the whole camera up and down the vertical graduated support (fig. 200).

§ **459. Focusing glass.** — There are two ways of using this:

1. A clear screen is used instead of a ground-glass. On this is a diamond scratch in the middle. The focusing glass is carefully focused on the central scratch, which must be in the exact plane where the sensitized photographic surface will be during the exposure. If now an object is brought to an accurate focus at this plane, it will also be in focus on the sensitized surface of the dry plate. Except for aid in arranging the object and for general focusing, the frosted glass can be entirely omitted, and a focusing glass giving about 8 to 10 diameters magnification is set in a board which takes the place of the ordinary frosted glass screen. This is put at the level to bring the focus exactly at the plane where the sensitive surface of the negative is to be.

The position of the focusing glass is determined as follows:

The plate holder with a clear glass plate or a thin negative is in the holder. And on the film side is a diamond scratch or an India ink mark near the middle of the face usually occupied by the sensitive film. It is very important that the mark should be on the side occupied by the film.

The scratch or ink mark is a guide for getting the focus at the right level. Now with a tripod or other magnifier, preferably with the magnifier to be used later, get the image focused of the metric scale, and its explanation or other sharp print exactly on the surface where the diamond or ink mark is. To make sure that there is no better focus obtainable, it is worth while to make a negative of the printed matter used for focusing. On the excellence of the focus determined depends the excellence of all future pictures which will be made. This method has the further advantage that the focus level is determined for the plate holder and not for a focusing screen. It is, in fact, an excellent way to check up the similarity of level of the ordinary focusing screen and the plate holder. Frequently they do not agree closely enough for the more exacting work, especially in photo-micrography. If the focus is found to be exact, proceed to set the focusing glass in a board as follows:

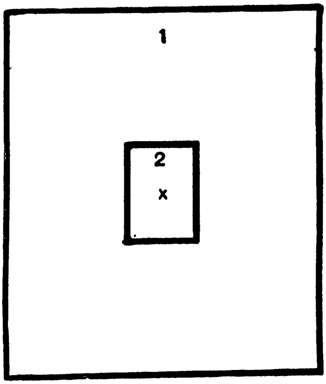


FIG. 201. GROUND-GLASS FOCUSING SCREEN WITH CLEAR CENTER FOR FINE FOCUSING.

1 The ground or frosted surface of the glass.

2 A cover-glass stuck to the frosted surface with *Canada Balsam*. This renders the frosted surface transparent.

x Pencil mark in the center of the focusing screen on the frosted surface to serve as guide when focusing with a magnifier.

Have a board of about 15 mm. thickness in a frame like that used for the ordinary focusing screen. Bore a hole in the center in which the focusing glass holder will fit snugly. Now put the frame on the focused camera and slowly twist the focusing glass into the hole until the focus seen through it is perfect. If nothing has changed in the camera, then this focus should give perfect results for any future setting of the camera, for the focus will be at the exact level occupied by the sensitive surface of the plate. If it is found perfect by trial, it is wise to put some shellac or other varnish around the mounting to fix it firmly in place in the wood so that there will be no change in its position. Of course, any change would result in imperfect, out-of-focus negatives.

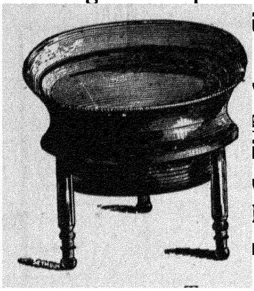


Fig. 202. Tripod Magnifier to Serve as a Focusing Glass.

This method of focusing has the great advantage of doing away with all obstructing glass. One focuses the position of the real image exactly as for a compound microscope when a positive ocular (figs. 22, 23) is used. It is an invaluable way for focusing in photomicrography.

§ **460. Objectives and magnification for embryos.** — It is a good plan to have one picture of natural size in each case, and then, if the embryos or other objects are very small, a picture of 5 or more times natural size. And a picture should go with the embryo or object throughout its entire career so that the exact appearance before sectioning or dissection will be available.

The objectives most convenient for making the photographs have an equivalent focus of from 50 to 150 mm. They are placed in the front board of the camera as usual (fig. 200). The larger the object the longer should be the focus of the objective; then the exaggerated perspective of short focused lenses will be avoided.

§ **461. Photographing bacterial cultures.** — For the successful photographing of these cultures dark-ground illumination is employed on the principle stated in § 171. That is, the preparation is illuminated with rays so oblique that none can enter the objective directly. Those striking the culture are reflected into the objective. The clear gelatin around the growth or colonies does not reflect the light, and therefore the space between the colonies is dark.

For supporting the Petri dishes a hole is made in a front board for the camera. This hole is slightly larger than the dish. Over it is then screwed or nailed a rubber ring slightly smaller than the Petri dish. This will stretch and receive the dish, and grasp it firmly, so that it is in no danger of falling out when put in a vertical position.

If the camera has two divisions the board with the Petri dish is put in the front of the camera, and the objective in the middle division through the side door. Otherwise the board holding the Petri dish must be on a separate support.

The vertical camera and focusing stand (fig. 200) lend themselves admirably for this kind of photography. The black background can be put on the lower stage and the Petri dish or other bacterial culture can be set on a glass plate or in a perforated board on the upper stage. The lighting is very easily accomplished by two portable lamps so arranged that no light can get directly from them into the objective.

One may use daylight by putting the culture in a support just outside a window, leaving the camera in the room. The rays from the sky are so oblique that they do not enter the objective. One must use a black, non-reflecting background some distance beyond the dish as in using artificial light (Atkinson).

In photographing bacterial cultures in test-tubes, the lighting is as in the preceding section, but a great difficulty is found in getting good results from the refraction and reflections of the curved surfaces. To overcome this one applies the principles discussed in § 341, and the test-tubes are immersed in a bath of water or water and glycerin. The bath must have plane surfaces. Behind it is the black velvet screen, and the light is in front, as for the Petri dishes. As suggested by Spitta, it is well to employ a bath sufficiently thick in order that streak cultures may be arranged so that the sloping surface will all be in focus at once by inclining the test-tube.

§ **462. Recording and storing negatives.** — Each negative should have a record upon it written on the film side with India ink; then it will never get mixed up. For ease in finding negatives there should be a record on the containing envelope also. Finally, it is a good plan to have a card catalogue of one's negatives. For a form see § 483.

For storing negatives a good method, where one does not have too many, is to put them in envelopes and store in boxes or drawers like book catalogue cards.

PHOTOGRAPHING WITH THE MICROSCOPE

§ **463.** The first pictures made on white paper and white leather, sensitized by silver nitrate, were made by the aid of a solar microscope (1802). The pictures were made by Wedgewood and Davy, and Davy says: "I have found that images of small objects produced by means of the solar microscope may be copied without difficulty on prepared paper" (§ 463a).

Thus among the very first of the experiments in photography the microscope was called into requisition. Naturally, plants and motionless objects were photographed in the beginnings of the art when the time of exposure required was long.

Although first in the field, photo-micrography has been least successful of the branches of photography. This is due to several causes. In the first place, microscope objectives have been constructed to give the clearest image to the eye; that is, the visual image, as it is sometimes called, is for microscopic observation of prime importance. The actinic or photographic image, on the other hand, is of prime importance for photography. For the majority of microscopic objects transmitted light (§ 70) must be used, not reflected light as in ordinary vision. Finally, from the shortness of focus and the smallness of the lenses, the proper illumination of the object is accomplished with some difficulty, and the fact of the lack of sharpness over the whole field with any but the lower powers has combined to make photo-micrography less successful than ordinary macro-photography. So tireless, however, have been the efforts of those who believed in the ultimate success of photo-micrography, that now the ordinary achromatic objectives with panchromatic or isochromatic plates and a color screen give good results, while the apochromatic objectives with projection oculars give excellent results, even in hands not especially skilled. The problem of illumination has also been solved by the construction of achromatic and apochromatic condensers and by the electric and other powerful lights now available. There still remains the difficulty of transmitted light and of so preparing the object that structural details stand out with sufficient clearness to make a picture which approaches in definiteness the drawing of a skilled artist.

The writer would advise all who wish to undertake photo-micrography seriously to study samples of the best work that has been produced. Among those who showed the possibilities of photo-micrographs was Col. Woodward of the U. S. Army Medical Museum. The photo-micrographs made by him and exhibited at the Centennial Celebration at Philadelphia in 1876 serve still as models. According to the writer's observation no photo-micrographs of histologic objects have ever exceeded those made by Woodward, and most of them are vastly inferior. It is gratifying to state, however, that at the present time many original papers are partly or wholly illustrated by photo-micrographs, and no country has produced works with photo-micrographic illustrations superior to those in Wilson's " Atlas of Fertilization and Karyokinesis " and Starr's " Atlas of Nerve Cells," issued by the Columbia University Press.

Most excellent photo-micrographs appear at frequent intervals in all the great biological journals. These should be studied by the young photographer ambitious to excel.

§ **463a.** Considerable confusion exists as to the proper nomenclature of photography with the microscope. On the Continent the term micro-photography (micro-photographie) is very common, while in English photo-micrography and micro-photography mean different things. Thus: A *photo-micrograph* is a photograph of a small or microscopic object usually made with a microscope and of sufficient size for observation with the unaided eye; while a *micro-photograph* is a small or microscopic photograph of an object, usually a large object, like a man or woman, and is designed to be looked at with a microscope.

Dr. A. C. Mercer, in an article in the Proc. Amer. Micr. Soc., 1886, p. 131, says that Mr. George Shadbolt made this distinction. See the Liverpool and Manchester *Photographic Journal* (now *British Journal of Photography*), Aug. 15, 1858, p. 203; also Sutton's Photographic Notes, Vol. III, 1858, pp. 205–208. On p. 208 of the last, Shadbolt's word "photo-micrography" appears. Dr. Mercer puts the case very neatly as follows: "A *Photo-Micrograph* is a *macroscopic* photograph of a *microscopic* object; a *micro-photograph* is a *microscopic* photograph of a *macroscopic* object." See also *Medical News*, Jan. 27, 1894, p. 108.

In a most interesting paper by A. C. Mercer on "The Indebtedness of Photography to Microscopy," Photographic Times Almanac, 1887, it is shown that: "To briefly recapitulate, photography is apparently somewhat indebted to microscopy for the first fleeting pictures of Wedgewood and Davy [1802], the first methods of producing permanent paper prints [Reede, 1837–1839], the first offering of prints for sale, the first plates engraved after photographs for the purpose of book illustration [Donne & Foucault, 1845], the photographic use of collodion [Archer & Diamond, 1851], and finally, wholly indebted for the origin of the gelatino-bromide process, greatest achievement of them all" [Dr. R. L. Maddox, 1871]. See further for the history of Photo-micrography, Neuhauss, also Bousfield, and Photography, 1839–1937. Museum of Modern Art, N. Y.

§ **464.** As the difficulties of photo-micrography are so much greater than of ordinary photography, the advice is almost universal that no one should try to learn photography and photo-micrography at the same time, but that one should learn the processes of photography by making portraits, landscapes, copying drawings, etc.; and then when the principles are learned, one can take up the more difficult subject of photo-micrography with some hope of success.

The advice of Sternberg is so pertinent and judicious that it is reproduced: " Those who have had no experience in making photo-micrographs are apt to expect too much and to underestimate the technical difficulties. Objects which under the microscope give a beautiful picture which we desire to reproduce by photography may be entirely unsuited for the purpose. In photographing with high powers it is necessary that the objects to be photographed be in a single plane and not crowded together and overlying each other. For this reason photographing bacteria in sections presents special difficulties and satisfactory results can be obtained only when the sections are extremely thin and the bacteria well stained. Even with the best preparations of this kind much care must be taken in selecting a field for photography. It must be remembered that the expert microscopist, in examining a section with high powers, has his finger on the fine adjustment screw and focuses up and down to bring different planes into view. He is in the habit of fixing his attention on the part of the field which is in focus and discarding the rest. But in a photograph the part of the field not in focus appears in a prominent way, which mars the beauty of the picture."

APPARATUS FOR PHOTO-MICROGRAPHY

§ **465. Camera.** — For the best results with the least expenditure of time one of the cameras especially designed for photo-micrography is desirable, but is not by any means indispensable for doing good work (fig. 200).

The first thing to do is to test the camera for the coincidence of the plane occupied by the sensitive plate and the ground-glass or focusing screen. Cameras even from the best makers are not always correctly adjusted.

For the method of procedure see above, § 459.

The majority of photo-micrographs do not exceed 8 centimeters in diameter and are made on plates 8 × 11, 10 × 13, or 13 × 18 centimeters ($3\frac{1}{4} \times 4\frac{1}{4}$ in., 4 × 5 in., or 5 × 7 in.).

For pictures larger than these it is best to make small, very sharp negatives of moderate enlargement and then print these at any desired size by means of projection apparatus. (See under enlargements, § 484).

§ **466. Workroom.** — It is almost self-evident that the camera must be in some place free from vibration. A basement room where the camera table may rest directly on the cement floor or on a pier is excellent. Such a place is almost necessary for the best work with high powers. For those living in cities, a time must also be chosen when there are no heavy vehicles moving in the streets. For less difficult work an ordinary room in a quiet part of the house or laboratory building will suffice. It helps much to have rubber corks in the lower ends of the table legs. The legs may also be made to stand on four thick pads of rubber or of thick felt. Finally the camera and microscope can be placed on a board platform and that put into a shallow box nearly filled with sawdust or dry sea sand.

The photo-engravers have overcome vibrations by suspending their cameras, or using spring coils as a part of the support. In case of real need this method would serve the photographer with the microscope.

§ **467. Arrangement and position of the camera and the microscope.** — For much photo-micrography a vertical camera and microscope are to be preferred. Excellent arrangements were perfected long ago, especially by the French. (See Moitessier).

Vertical photo-micrographic cameras are now commonly made, and by some firms only vertical cameras are produced. They are exceedingly convenient, and do not require so great a disarrange-

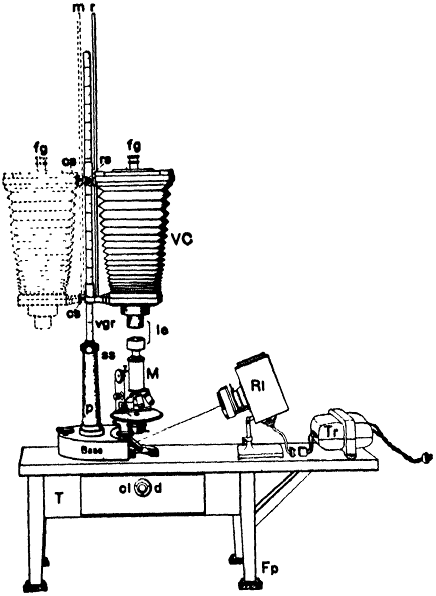


FIG. 203. VERTICAL MICROSCOPE AND CAMERA FOR PHOTO-MICROGRAPHY. (About $\frac{1}{10}$ natural size).

T Low table 50 cm. high, 50 cm. wide, and 70 cm. long with felt pads under the legs (*fp*) and a drawer with combination lock (*cl.d*).

M Microscope.

VC Vertical camera supported by the revolving rod (*vgr*) which is graduated in centimeters and half centimeters. The camera may be turned aside as shown by the dotted lines.

Base The heavy iron base and pillar (*p*) supporting the revolving rod (*vgr*), which in turn supports the camera.

cs Clamping screws to fix the two ends of the camera in any desired position.

mr Magnification rod. This serves to hold the extension of the camera at the right point for any desired magnification; then the camera as a whole moves up and down on the graduated rod (*vgr*).

rs Clamp to fix the camera at any desired extension on the magnification rod (*mr*).

fg Focusing glass (§ 459).

le Light excluder (fig. 204–205).

Rl Research lamp with 108-watt bulb, and transformer (*Tr*). For full description see fig. 80.

ment of the microscope to make the picture as do the horizontal ones. The variation in size of the picture in this case is mostly obtained by the objective and the projection ocular rather than by length of bellows.

It must not be forgotten, however, that penetration varies inversely as the numerical aperture, and inversely also as the *square of the power*. There is then an advantage in using a low power with long bellows if one needs penetration. In many cases the best way is to use a moderate power and a short bellows, and then to print the negative as for making enlargements for drawings (§ 484).

For convenience and rapidity of work a microscope with mechanical stage is necessary; and for sections where it is desirable to have the image in some regular position a revolving stage to the microscope helps greatly in orienting the image on the plate.

It is also an advantage to have a tube of large diameter so that the field will not be too greatly restricted (fig. 179). In some microscopes the tube is removable almost to the nose-piece to avoid interfering with the size of the image. The substage condenser should be movable on a rack and pinion. The microscope should have a flexible pillar for work in a horizontal position. While it is desirable in all cases to have the best and most convenient apparatus that is made, it is not by any means necessary for the production of excellent work. A simple stand with flexible pillar and good fine adjustment will answer.

§ 468. Objectives and oculars for photo-micrography. — The belief is almost universal that the apochromatic objectives are most satisfactory for photography. They are employed for this purpose with a special projection ocular or compensation oculars. Two low powers are used without any ocular. Some of the best work that has ever been done, however, was done with achromatic objectives (work of Woodward and others). One need not desist from undertaking photo-micrography if he has good achromatic objectives. From a somewhat extended series of experiments with the objectives of many makers the modern fluorite and achromatic objectives have been found to give excellent results when used without an ocular. Most of them also gave good results with projection and other oculars.

Recently negative lens combinations (Homals, Ampliplans) have been devised to use instead of oculars for photo-micrography and for projection. They are in the nature of amplifiers (§ 369) and serve to compensate for curvature of the field and for chromatic differences of magnification of the objectives. (See Bausch & Lomb's Catalog of Scientific Instruments, 1941, p. 146.)

§ **469. Difference of visual and actinic foci.** — Formerly there was much difficulty experienced in photo-micrographing on account of the difference in actinic and visual foci. Modern objectives are less faulty in this respect and the apochromatics are practically free from it. Since the introduction of orthochromatic or isochromatic and panchromatic plates, and in many cases the use of color screens, but little trouble has arisen from differences in the foci. This is especially true when mono-chromatic light and even when petroleum light is used. In case an objective has its visual and actinic foci at markedly different levels, it would be better to discard it for photography altogether, for the estimation of the proper position of the sensitive plate after focusing is only guesswork and the result is mere chance. If sharp pictures cannot be obtained with an objective when isochromatic or panchromatic plates are used, the fault may not rest with the objective, but with the plate holder and focusing screen. They should be very carefully tested to see if there is coincidence in position of the focusing screen and the sensitive film as described in § 459.

LIGHTING FOR PHOTO-MICROGRAPHY

§ **470. Light.** — The best light is sunlight. That has the defect of not always being available, and of differing greatly in intensity from hour to hour, day to day, and season to season. Following the sunlight the electric light is the most intense of the available lights. (See also figure 129 A and § 499.)

As natural daylight is not constantly available, the photo-micrographer has now at his disposal the artificial daylight by the use of a nitrogen-filled mazda lamp and daylight glass. The lantern for this shown in figs. 80, 203 was found to be excellent and the results obtained by its use in photographing with powers up to the 1.5 mm. (115x) homogeneous immersion were good. Of course any light filters which are adapted to natural daylight would serve perfectly with the artificial daylight. In most cases the six-volt lamp requires a special filter for each specimen. See § 491.

For preparations needing a yellow color screen for daylight, a petroleum or kerosene lamp gives good results for the majority of low and moderate power work. And even for 2 mm. (90x) homogeneous immersion objectives, the time of exposure is not excessive for many specimens (40 seconds to 3 minutes).

A lamp with flat wick about 40 mm. wide has been found most generally serviceable. For large objects and low powers the flame may be made large and the face turned toward the mirror. This will light a large field. For high powers the edge toward the mirror gives an intense light. The ordinary glass chimney answers well, especially where a shield is used.

In managing the light for photography with the microscope, follow the directions in § 198. See below for the use of color screens (§ 491).

§ **471. Objects suitable for photo-micrographs.** — While almost any large object may be photographed well with the ordinary camera and photographic objective, only a small part of the objects mounted for microscopic study can be photo-micrographed satisfactorily. Many objects that can be seen clearly by constant focusing with the fine adjustment, appear almost without detail on the screen of the photo-micrographic camera and in the photo-micrograph.

If one examines a series of photo-micrographs, the chances are that the greater number will be of diatoms, plant sections, or preparations of insects. That is, they are of objects having sharp details and definite outlines, so that contrast and definiteness may be readily obtained. Stained microbes also furnish favorable objects when mounted as cover-glass preparations, but these give color images and require a color screen.

For success with preparations of animal tissue they must approximate as nearly as possible to the conditions more easily obtained with vegetable preparations. That is, they must be made so thin and be so prepared that the cell outlines have something of the definiteness of vegetable tissue. It is useless to expect to get a clear photograph of a section in which the details are seen with difficulty when studying it under the microscope in the ordinary way.

Many sections which are unsatisfactory as wholes may nevertheless have parts in which the structural details show with satisfactory clearness. In such a case the part of the section showing details satisfactorily should be marked in some way. If one's preparations have been carefully studied and the special points in them indicated, they will be found far more valuable both for ordinary demonstration and for photography. The amount of time saved by marking one's specimens can hardly be overestimated.

Formerly many histologic preparations could not be satisfactorily photographed. Now with improved section cutters, better staining and mounting methods, and with the color screens and isochromatic and panchromatic plates (§ 505) almost any preparation which shows the elements clearly when looking into the microscope can be satisfactorily photographed. Good photographs cannot, however, be obtained from poor preparations by any method.

In photo-micrography do not forget the three ways in which details of structure may be brought out clearly:

(1) By difference of refraction of the object and the mounting medium (refraction images, § 152).

(2) By differential staining (color images, § 154).

(3) By means of dark-ground illumination.

EXPERIMENTS IN PHOTO-MICROGRAPHY

§ **472.** The following experiments are introduced to show practically just how one would proceed to make photo-micrographs with various powers, and be reasonably certain of fair success. If one consults prints or the published figures made directly from photo-micrographs, it will be seen that, excepting diatoms and bacteria, the magnification ranges mostly between 10 and 150 diameters.

§ **473. Focusing in photo-micrography.** — For rough focusing and as a guide for the proper arrangement of the object one uses a ground-glass screen, as in gross photography. With the ground-glass screen one can judge of the brilliancy and evenness of the illumination more accurately than in any other way. For final and exact focusing two principal methods are employed:

(*a*) A focusing glass is used either with a clear screen or in a board screen, as described above (§ 459). The latter method is like focusing with the compound microscope and a positive ocular. If the focusing glass is set properly the focus should be easily and accurately determined.

In whatever way one focuses for photo-micrography a difficulty often appears. No matter how perfect the focus of the microscope, the picture may be out of focus. This may be due to either one of two things: (1) the focusing screen or focusing glass may not be in the right position to make the image sharp on the sensitive plate; (2) the microscope may get out of focus while the picture is being made. The reason for this change may be the gradual settling down of the tube of the microscope. This may be a fault of the fine or of the coarse adjustment. It is a good plan to focus the object carefully and, after 10 or 15 minutes, to see if the focus is still good. If the microscope will not stay in focus, one cannot get a good picture. In that case it is necessary to study the apparatus and see which part of the mechanism is at fault.

§ **474. Photo-micrographs of 20 to 50 diameters.** — For pictures under 10 to 15 diameters it is better to use the camera for embryos with the objective in the end of the camera, and the special microscope stand for focusing (fig. 200).

For pictures at 25 to 50 diameters one may use the microscope with a low objective, 20 (8x) to 35 mm. (4x) equivalent focus, and no ocular (fig. 179). The object is placed on the stage of the microscope and focused as in ordinary observation. If a vertical microscope is used, the light from the petroleum lamp or other artificial light is reflected upward by the mirror. It may take some time to get the whole field lighted evenly. In some cases it may be advisable to discard the condenser and use the mirror only. For some purposes one will get a better light by placing the bulls'-eye or other condenser between the lamp and the mirror to make the rays parallel, or even to make a sharp image of the lamp flame on the mirror. Remember also that in many cases it is necessary to have a color screen between the source of light and the object (§§ 491–495).

For a horizontal camera it is frequently better to swing the mirror entirely out of the way and allow the light to enter the condenser directly. When the light is satisfactory, as seen through an ordinary ocular, remove the ocular.

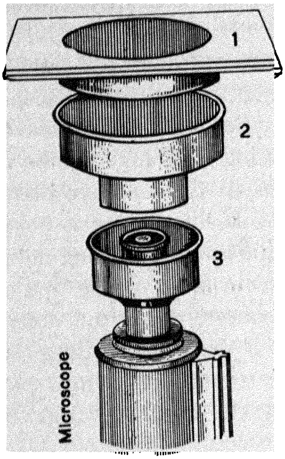


Fig. 204. Light Excluder for Connecting the Camera and Microscope (Zeiss form) (About ¼ natural size).

1 The front board of the camera.

2 Connecting piece to fit over *1* and extend down into *3*.

3 Piece to fit over the upper end of the tube of the microscope and to receive the lower end of *2* (compare fig. 205 where the parts are together as in making an exposure).

(*a*) *Photographing without an ocular.* — After the removal of the ocular put in the end of the tube a lining of black velvet to avoid reflections. Connect the microscope with the camera, making a light-tight joint, and focus the image on the focusing screen. One may make a light-tight connection by the use of black velveteen or, more conveniently, by the double metal hood which slips over the end of the tube of the microscope, and into which fits a metal cylinder on the lower end of the camera (figs. 204–205). In figure 205 the connection has been made.

It will be necessary to focus down considerably to make the image clear. Lengthen or shorten the bellows to make the image of the desired size, then focus with the utmost care. In case the field is too much restricted on account of the tube of the microscope, remove the draw-tube. When all is in readiness, it is well to wait for three to five minutes and then to see if the image is still sharply focused. If it gets out of focus simply by standing, a sharp picture cannot be obtained. If it does not remain in focus, something is faulty. When the image remains sharp after focusing, make the exposure. From 20 to 60 seconds will usually be sufficient time with medium plates and light as described. If a color screen is used it will require 50–300 seconds, i.e., 2 to 5 times as long, for a proper exposure (§ 497).

(*b*) *Photographing with an ocular.* — If the object is small enough to be included in the field of a projection or other ocular (fig. 208), use that for making the negative as follows: Swing the camera around so that it will leave the microscope free (fig. 203). Use an ordinary ocular, focus and light the object, then insert a projection ocular in place of the ordinary one, and swing the camera back over the microscope. It is not necessary to use an ordinary ocular for the first focusing, but as its field is larger, it is easier to find the part to be photographed. The first step is then to focus the diaphragm of the projection ocular sharply on the focusing screen. Bring the camera up close to the microscope and then screw out the eyelens of the ocular a short distance. Observe the circle of light on the focusing screen to see if its edges are perfectly sharp. If not, continue to screw out the eyelens until it is. If it cannot be made sharp by screwing it out, reverse the operation. Unless the edge of the light circle, i.e., the diaphragm of the ocular, is sharp, the resulting picture will not be satisfactory.

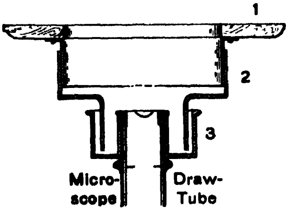


FIG. 205. LIGHT EXCLUDER FOR PHOTO-MICROGRAPHY.

(About ½ natural size).

In this figure the different parts of the light excluder are in position for making an exposure.

1 The front board of the camera.

2 The intermediate part connecting the camera and the hollow cylinder on the upper end of the microscope draw-tube. (Compare fig. 204).

It should be stated that for the 2x projection ocular the bellows of the camera must be extended about 30 or 40 centimeters or the diaphragm cannot be satisfactorily focused on the screen. The 4x projection ocular can be focused with the bellows much shorter. For either projection ocular the screen distance can be extended almost indefinitely.

When the diaphragm is sharply focused on the screen, the microscope is focused, that is, first with the unaided eye then with the focusing glass. The exposure is made in the same way as though no ocular were used (§ 474a), although one must have regard to the greater magnification produced by the projection ocular and increase the time accordingly; thus, when the 4x ocular is used, the time should be at least doubled over that when no ocular is employed. The time will be still further increased if a color screen is used (§ 501).

It is recommended that when the bellows have sufficient length the lower projection oculars be used, but with short bellows the higher ones.

§ **475. Magnification of a photomicrograph.** — This is easily determined by removing the specimen and putting in its place a stage micrometer in 1/10th and 1/100th mm. spaces. If the image of the micrometer is focused sharply one can measure the image of the spaces between the lines. By dividing the size of the image by the known size of the space the quotient will be the magnification. For example, if the micrometer spaces used are 1/10th mm. and the image of one space measures 12 mm. the magnification is 120 (12 ÷ 1/10th = 120). With this method one must make the determination for every photomicrograph taken unless a camera of fixed bellows length is employed.

The following procedure is more satisfactory and much less trouble in the long run. The extensible bellows of the camera is clamped at some definite length, then negatives are made of the stage micrometer with the different objectives and oculars which are to be used in photographing. The magnification for each combination is determined as indicated above and marked on the negative. When a photographic negative of some microscopic object is made with the same combination one can see at once the magnification by consulting the micrometer negative.

This method has a further advantage if one wishes the photomicrographic print or diagram made from the original negative to be of greater or less magnification than the original. For example, as in the case above where the magnification is 120, suppose the print desired is to be at 350 magnification. The negative of the micrometer is put in the enlarging apparatus and the distance found by trial where the image of 1/10th mm. projected on the screen measures 35 mm. (35 ÷ 1/10 = 350). If now one puts the negative of the microscopic object at a magnification of 120 in place of the micrometer negative, its image will be 350 diameters on the screen, and if a print or diagram is made at that screen distance the picture will be at a magnification of 350. Suppose on the other hand that a classroom diagram is desired at a magnification of 10,000 diameters of this same negative. As before the negative of the micrometer in 1/10ths and 1/100ths mm. is put in the enlarging apparatus and moved to a distance from the screen so that when in sharp focus the image of 1/100th mm. measures on the screen 100 mm., the magnification will then be as desired (100 ÷ 1/100th = 10,000), and if the negative of the object at 120 magnification is diagrammed at this distance the magnification will be 10,000.

If a reduced image is desired, it is likewise simple; e.g., if a print of exactly 100 diameters is desired from this same negative it can be obtained by using the micrometer negative and getting the image of 1/10th mm. to measure exactly 10 mm. on the screen (10 ÷ 1/10th = 100).

§ **476. Photo-micrographs at a magnification of 100 to 150 diameters.** — For this, the simple arrangements given in the preceding section will answer, but the objectives must be of shorter focus, 8 to 3 mm.

§ 477. Lighting for photo-micrography with moderate and high powers. (100 to 2500 diameters). — No matter how good one's apparatus, successful photo-micrographs cannot be made unless the object to be photographed is properly illuminated. The beginner should go over with the greatest care the directions for centering the condenser, for centering the source of illumination, and the discussion of the proper cone of light and lighting the whole field, as given in §§ 135-137. Then for each picture he must take the necessary pains to light the object properly. An achromatic condenser is almost a necessity (§ 128). Whether a color screen should be used depends upon judgment and that can be attained only by experience. In the beginning one may try without a screen and with different screens and compare results.

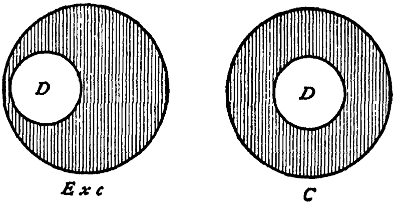


FIG. 206. VIEW OF THE BACK LENS OF THE OBJECTIVE, SHOWING THE CONDENSER OUT OF CENTER AND CENTERED.

Exc The spot of light (*D*) is to one side of the center, showing that the optic axis of the condenser is not in line with that of the microscope.

C The spot of light (*D*) is in the center, showing that the optic axis of the condenser and microscope are in line.

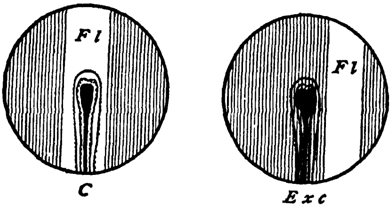


FIG. 207. FIELD OF THE MICROSCOPE SHOWING THE LIGHT IN THE CENTER AND TO ONE SIDE

C Fl The light is in the center and illuminates the object.

Exc Fl The light is at one side of the center and does not illuminate the object. (The field is not fully lighted, as a low power is used to center the object and the light).

A plan used by many skilled workers is to light the object and the field around it well, and then to place a metal diaphragm of the proper size in the camera just under the plate holder. This will insure a clean, sharp margin to the picture. This metal diaphragm must be removed while focusing the diaphragm of the projection ocular, as the diaphragm opening is smaller than the image of the ocular diaphragm.

If the young photo-micrographer will be careful to select for his first trials objects of which really good photo-micrographs have already been made, and then persists with each one until fairly good results are attained, his progress will be far more rapid than as if poor pictures of many different things were taken. He should, of course, begin with low magnifications.

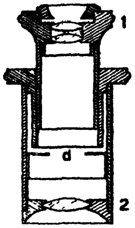


FIG. 208. PROJECTION OCULAR IN SECTION.
(About ½ natural Size).

1 The upper or eye end of the ocular; it is composed of two convex and one concave lens and serves to project the real image formed by the objective and field lens at *d* upon the screen or photographic plate. It is movable to permit of focusing at different screen distances.

2 Field lens of the projection ocular.

d Diaphragm where the real image is formed.

§ **478. Adjusting the objective for cover-glass.** — After the object is properly lighted, the objective, if adjustable, must be corrected for the thickness of cover. If one knows the exact thickness of the cover and the objective is marked for different thicknesses, it is easy to get the adjustment approximately correct mechanically; then the final corrections depend on the skill and judgment of the worker. It is to be noted, too, that if the objective is to be used without a projection ocular, the tube-length is extended practically to the focusing screen, and as the effect of lengthening the tube is the same as thickening the cover-glass, the adjusting collar must be turned to a higher number than the actual thickness of the cover calls for (see § 149).

§ **479. Photographing without an ocular.** — Proceed exactly as described for the lower power, but if the objective is adjustable, make the proper adjustment for the increased tube-length (§ 149).

§ **480. Photographing with a projection ocular.** — Proceed as described in § 474b, only in this case the objective is not to be adjusted for the extra length of bellows. If it is corrected for the ordinary ocular, the projection ocular then projects this correct image upon the focusing-screen.

§ **481. Photo-micrographs at a magnification of 500 to 2000 diameters.** — For this the homogeneous immersion objective is employed, and as it requires a long bellows to get the higher magnification with the objective alone, it is best to use the projection oculars. Compensation oculars may also be used.

For this work the directions given in §§ 135–137 must be followed with great exactness. The edge of the petroleum lamp flame is sufficient to fill the field in most cases. With many objects the time required with good lamplight is not excessive; viz., 2 to 3 seconds. The reason for this is that while the illumination diminishes directly as the square of the magnification, it increases with the increase in the numerical aperture, so that the illuminating power of the homogeneous immersion is great in spite of the great magnification.

For work with high powers a stronger light than the petroleum lamp is employed by those doing considerable photo-micrography, e.g., the arc light or the 108-watt, 6-volt lamp (figs. 80, 128).

It may be well to recall the statement made in the beginning, that the specimen to be photographed must be of special excellence for all powers. No one who undertakes to make photomicrographs at a magnification of 500 to 2000 diameters will doubt the truth of the statement.

If one has a complete outfit with electric arc light or the 108-watt lamp, the time required for photographing objects is much reduced, i.e., ranging from 1 to 20 seconds even with the color screen. As the light is so intense with the arc light it is necessary to soften it greatly for focusing. Several thicknesses of ground-glass placed between the lamp and the microscope will answer. These are removed before taking the negative. It is well also to have a water bath on the optical bench to absorb the radiant heat. This should be in position constantly (figs. 179, 180).

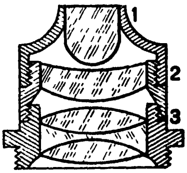


FIG. 209. ACHROMATIC SUBSTAGE CONDENSER FOR PHOTO-MICROGRAPHY.

(From Watson's Catalogue).

1, 2, 3 The three optical parts of the condenser. (Compare figs. 60–61 also the construction of objectives in figs. 19–21 and note that the condenser is like an inverted objective.)

§ **482. Use of oculars in photo-micrography.** — There is much diversity of opinion whether or not the ordinary oculars used for observation should be used in photographing. Excellent results have been obtained with them and also without them.

When an ocular is used, the eyelens serves to project a real image of the objective, not to act as a magnifier with the eye as an ordinary observation; therefore for the best results in photography this eyelens should be a combination which will give a correct image. For apochromatic objectives the projection or the compensation oculars should be used, not ordinary Huygenian oculars. The projection and compensation oculars work well with the best high-angled achromatic objectives also.

§ 483. Negative record in photography. —

Name No. Location

Camera..........................	Date............................
....	Exposure........................
Objective.......................	Developer.......................
Ocular.........................	Fixer ....
Condenser.......................	Mag. ×..........................
Diaphragm.......................	Remarks.........................
Object stained with.............	....
Color screen....................	....
Plate...........................	....
Light and hour..................	....
....	....

Projection Apparatus for Photographic Enlargements

§ 484. Enlarged prints of small negatives. — There is great advantage in making pictures of large objects at a considerable distance with a long-focus objective, so that the perspective will be correct and all levels of the object will be in good focus. It is also advantageous to make pictures of microscopic objects without undue enlargement; then there is greater sharpness of the object as a whole.

If now one wishes a large print, any good negative can be used and a print obtained of almost any desired enlargement by using a photographic objective for projecting the image upon the photographic paper. This is done with projection apparatus in a dark room as follows: The management of the projection apparatus is as for drawing. The negative is placed in some kind of holder and put in the cone of light of the main condenser where the part of it to be enlarged is fully illuminated. An erect image will be printed on the paper if the film side of the negative faces the sensitive paper exactly as for contact printing. Of course, if it is desired to reverse the position, it can be done by turning the film side toward the source of light.

§ **485. Size of condenser required.** — The general law is that the diameter of the condenser must be equal to or somewhat greater than the diagonal of the negative or part of the negative to be enlarged. For example, to enlarge the whole of a lantern slide negative (85 × 100 mm.), the condenser should have a diameter of 14 cm. For a negative 100 × 125 mm. the condenser should be 18 cm. in diameter; for one 125 × 175 mm. the condenser should be 23 cm. in diameter; and for a negative 200 × 250 mm. the condenser should be 35 cm. in diameter.

§ **486. Objectives to use for enlarging.** — It is necessary to use an objective which has been corrected for photography. The ordinary projection objective gives a good visual image, but not a good photographic image. The iris diaphragm must be wide open (§ 487).

In preparing for printing, which, of course, is done in a dark room, put some white paper in a printing frame with a clear glass in it. Hold it in the path of the beam from the projection apparatus, and either by moving a support near the apparatus, or by moving the projection apparatus, get the desired size of picture. One can determine the exact magnification by putting a lantern slide of the metric scale (fig. 173) in place of the negative and projecting its image upon the white paper in the printing frame.

§ **487. Focusing and printing.** — Focus the image of the negative as sharply as possible. Then put over the end of the objective a cover of some kind with ruby glass in it. This will allow the light to pass in part, but it will not affect the photographic paper to be used.

Place in the printing frame some developing paper like vitava rapid black or velox. Place the printing frame in position. The image will show clearly on the paper by the red light. When the frame is in the exact position desired, remove the cap with ruby glass and make the exposure. With an arc light the time will vary from about 1 to 10 seconds, depending on the density of the negative. Cover the objective, turn off the arc lamp and develop the print as for contact printed pictures. A mazda lamp may be used instead of an arc light for enlarging. If the rather large source of light in the 110-volt lamp is used, a diffuser of ground glass is needed to avoid the shadows between the filaments. When a diffuser is used with the mazda or arc light, the diaphragm of the objective can be closed as much as desired, but of course it then takes a much longer exposure. If now one uses a 6-volt mazda head-light lamp by inserting a transformer in the circuit for the alternating current, or by using a storage battery for the direct current, the filament is so concentrated that the source may be treated like that of an arc light, and no diffuser used. This makes it possible to use the full opening of the objective. The candle power of the 6-volt mazda is much less than that of the arc light, but it has the advantage of requiring no attention after being once centered (figs. 79–82).

§ 488. Printing the image of an object directly on the paper. — With the apparatus set up exactly as for drawing or for printing enlargements, one can expose the developing photographic paper to the sharply focused image of the specimen. Of course this will give a negative image, all the lights and shades being reversed, but the outlines and proportions are perfect. Such pictures serve as useful a purpose as shade-correct pictures for model making and for keeping a record of one's specimens.

Photographic Representation of Visual Appearances; Panchromatic Photography with Color Screens

§ 489. Five methods of rendering objects visible. —

(1) The mounting medium and the object must have different refractive indices, then the outline of the object or of its details are margined by dark borders (§ 152, refraction images).

(2) The object or its details must have a different color from the surrounding medium or neighboring objects (color images, § 154).

(3) The object or its details must appear self-luminous, the surrounding field being dark (method of dark-ground illumination or ultra-violet radiation with resulting fluorescence).

(4) If reflected light is used, some parts of the object must absorb the light and some parts reflect it; the different parts will then appear as light and dark.

(5) If transmitted light is used, some parts of the object must be transparent or translucent and other parts opaque. The opaque parts will then appear dark, and the transparent or translucent parts light.

Two, four and five might properly be called absorption images.

§ **490.** Photography is admirably adapted to represent the visual appearances of both naked eye and microscopic objects. There is only one difficulty which is really serious, and that is in the proper representation in black and white of the various colors.

This difficulty is inherent in the sensitiveness of the eye to colors and the unlike sensitiveness of the photographic plate to the same colors. If both were equally and similarly sensitive, then the photographic representation of color in shades or tones of black and white would have the same brightness as the different colors to the eye. But the eye has its maximum sensitiveness in the green (fig. 210), while the photographic plate has almost all of its sensitiveness in the violet-blue end of the spectrum. Indeed it is sensitive to a part of the ultra violet which is wholly dark to the eye. Hence the photograph represents the brilliant red-orange-yellow-green image seen by the eye as dark, while the relatively dark violet-blue to the eye is rendered white by the photographic plate. The photographic image of colored objects is then a kind of negative of the same image to the eye. This has made the use of photography unsatisfactory where objects have color, and most objects in nature are colored more or less; and one of the greatest triumphs of microscopic science has been the differentiation of details of structure by selective staining.

From the earliest history of photography the inability to render the colors properly or in actual colors has been greatly deplored. To give the proper brightness in tones of black and white to colored objects, two things had to be attained:

(1) The photographic plates, which were originally sensitive only to the violet-blue end of the spectrum, had to be rendered sensitive to the other colors. The first step was in getting plates sensitive to the spectrum as far as the yellow. These are the so-called isochromatic or orthochromatic plates. The final step was to get plates sensitive to all the colors of the spectrum, including the orange and red. These are known as panchromatic or spectrum plates.

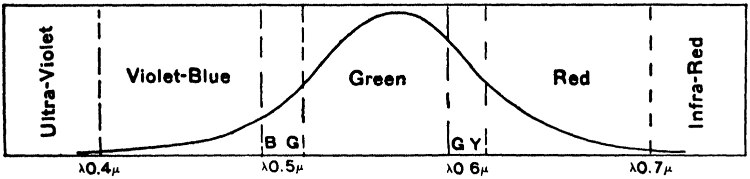


FIG. 210. SENSITIVENESS OF THE EYE TO THE SPECTRUM WITH MODERATE ILLUMINATION.

(Base Lines = Wave lengths × 250,000 times).

As shown in this curve the normal human eye with moderate illumination has its maximum sensitiveness at about wave length λ0.55μ, that is, in the green next the yellow. With very brilliant light the greatest sensitiveness is in the yellow, while with dim light it moves along well into the green. (See § 288 for designation of wave lengths in microns, etc.).

Ultra-violet Short radiation invisible to the eye. Compare the sensitiveness of the photographic plate to this radiation (fig. 211–213).

Violet-blue Radiation at the blue end of the spectrum.

Green Radiation in the middle of the spectrum.

Red Radiation at the red end of the spectrum.

Infra-red Long radiation invisible to the eye.

G Y Borderland between green and yellow.

B G Borderland between blue and green.

(2) But as all of these color-sensitive plates are more sensitive to the violet-blue than to the other colors, it is necessary to use some means for reducing or blocking out part of the violet-blue light without interfering with the action of the other colors (§ 492). For gaining contrast effects it was necessary to devise means for blocking out special parts of the spectrum (§ 493). These selecting media are known as color screens or ray filters.

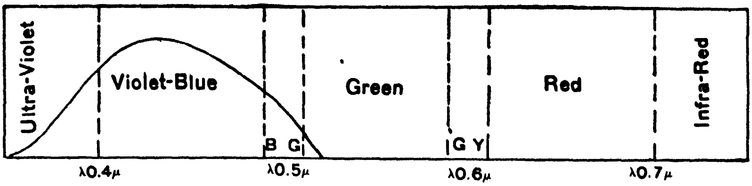


FIG. 211. NORMAL SPECTRUM SHOWING THE SENSITIVENESS OF ORDINARY PHOTOGRAPHIC PLATES.

(After Mees; and magnified as in fig. 210).

As shown in this curve, the ordinary photographic plate is sensitive only in the blue end of the spectrum including the ultra-violet, the maximum sensitiveness being at about wave length λ0.45μ. It is insensitive to all wave lengths longer than about λ0.52μ. (Compare with fig. 210, 212–213).

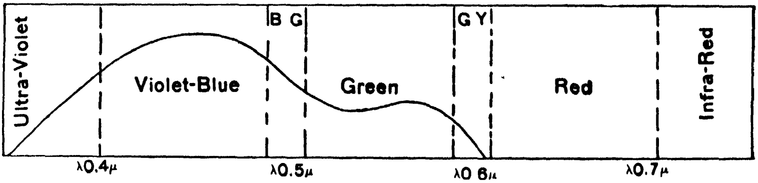


FIG. 212. NORMAL SPECTRUM SHOWING THE SENSITIVENESS OF ORTHOCHROMATIC OR ISOCHROMATIC PLATES.

(After Mees; magnification as in fig. 210).

These plates have practically the same sensitiveness as the ordinary plates except that the sensitiveness is continued through the green and yellow. (Compare figs. 210, 211 and 213).

COLOR SCREENS OR RAY FILTERS

§ **491. Color screens or ray filters.** — These are transparent, colored bodies which select the wave lengths of light which they transmit and absorb the other waves, or they diminish more or less some of the wave lengths and transmit the others with very slight loss. The color of such a screen to the eye will be determined by the light which it transmits in the greatest quantity. For example, if the violet-blue light is absorbed, the remaining light will appear yellow, while if green and red are absorbed the transmitted light will appear blue; if violet-blue and green are absorbed, the light will appear red; and if violet-blue and red are largely absorbed; the remaining light will appear green.

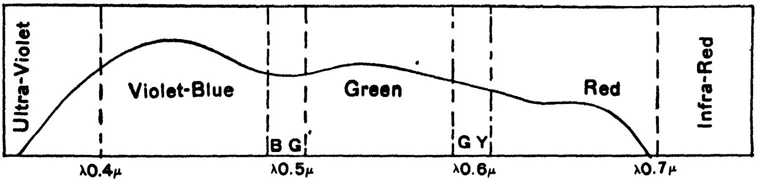


Fig. 213. Normal Spectrum Showing the Sensitiveness of Panchromatic Plates.

(After Mees; magnification as in fig. 210).

Panchromatic plates have the maximum sensitiveness still in the violet-blue, but it is extended to include the red. (Compare figs. 210–212).

§ **492. Compensating ray filters.** — These are filters or screens which aid the panchromatic photographic plate in giving a black and white picture of colored objects which shall correspond in brightness to the different colors as seen by the eye.

As all photographic plates, even the panchromatic ones, are more sensitive to the violet-blue than to the other colors of the spectrum (fig. 213), the effect of the violet-blue must be reduced, hence yellow screens must be used to do this and compensate for the smaller sensitiveness of the plate for the other parts of the spectrum.

Fortunately the great photographic manufacturers have made a study of the principles of color screens as well as of their plates, and they supply workers with data showing what wave lengths of light their different plates are sensitive to, and what wave lengths are absorbed wholly or in part by their ray filters. They also give advice from abundant experience as to the proper combination of plate and color screen to get the best effect in photographing a great variety of colored objects. By using this information, and profiting by experience, one can learn to photograph almost any object successfully.

§ **493. Contrast ray filters.** — These are filters or screens by the aid of which strong contrasts in black and white are given to various colored objects or their details. As given in the general statement of the basis for visibility of objects and their details, refraction and opacity are of prime importance for securing sharp outlines. Color images are also of the greatest advantage in differentiating the details of microscopic structure; but as color does not appear in the ordinary photograph, the differentiation of colored objects must be secured by producing shades of light and dark up to complete blackness in some cases. For example, in some microscopic specimens important details may be stained violet or blue. To the eye these violet or blue objects stand out with great clearness. In the photograph, on the other hand, without special help from a color screen, they are wholly lost or are so faint that they can hardly be seen. To make such details stand out in shades of black, a yellow color screen absorbing violet-blue and allowing the other colors to pass is used with a plate sensitive to the other colors to be photographed. A picture is thus obtained which shows the violet-blue objects in black and the other details in various shades.

A contrast color screen does not, of course, give correct brightness, but the purpose in using it is to bring out in the most striking manner the form of certain structures. The general law is: For contrast effects, use a color screen which absorbs the light transmitted normally by the colored object, but allows the other colors to pass.

§ **494. Refraction and opacity and color screens.** — It should not be forgotten in using color screens and color-sensitive plates that refraction and opacity exert their full effect in producing the final result. The color screen acts only to suppress or lessen certain definite wave lengths. Refraction and opacity tend to suppress all wave lengths in certain limited borders or definite areas. Hence any stain like hematoxylin which tends to make an object more opaque to all parts of the spectrum will increase the contrast even if no color screen is used.

§ **495. Lessening contrast.** — With some specimens it is necessary to lessen contrast in order to bring out details of structure. One of the striking examples frequently referred to is whalebone. A microscopic section of this has a reddish appearance by transmitted light. If now a blue screen is used with a panchromatic plate, the greatest possible contrast is obtained, and the object loses all detail in the photograph. If, on the other hand, a red screen is used, the photograph shows good detail and the general appearance is like that seen by the eye in looking into the microscope.

The general law is: When the contrast is too great, use a color screen of the same color as the object, and, of course, a plate must be used sensitive to that color.

§ **496. Use of the micro-spectroscope in photo-micrography.** — If one studies his specimens with the micro-spectroscope and makes sure exactly what light is transmitted by them, it will be possible to judge with intelligence what plate and what color screen to use to bring out in the most satisfactory manner their structural appearances. Fortunately the manufacturers furnish the information concerning their plates and the color filters, so that labor is spared the individual worker. It might be worth while for him to check up the color screens occasionally to make sure that they have not deteriorated.

§ **497. Time of exposure for photo-micrographs.** — This varies from the fraction of a second to several minutes, depending on four factors:

(1) The nature and intensity of the light.

(2) The magnification of the microscope. The higher the magnification, the longer must be the exposure.

(3) The transparency of the specimen. The more transparent, the shorter the exposures.

(4) The thicker or deeper the color of the ray filter, the longer must be the exposure.

(5) Red stains require longer exposure than blue stains.

§ **498. Daylight.** — This has served for some of the best photo-micrographs that have ever been made. Its great defect is that it is not always available.

§ **499. Artificial lights.** — Kerosene (petroleum) and now the electric lights (arc light, incandescent lamp) are most used. The 108-watt, ribbon-filament lamp is most convenient and suitable (§ 198).

The excess intensity in the red end of the kerosense light, and to a less degree of the electric illuminants, serves in part at least to compensate for the greater sensitivity of most photographic media to the violet-blue end of the spectrum. (See figures 211–213.)

For many purposes the line spectra of the high pressure, capillary mercury arc are of advantage, especially the blue, green and orange wave-lengths which with suitable screens or filters can be used separately or in combination, depending on the character of the object to be photographed (fig. 129 A).

Ultra-violet and infra-red radiations are also coming to have a prominent place in photo-micrography and in general photography. See especially the Kodak manuals in the collateral reading.

§ **500. Mutual adaptation of color screen and light.** — As the color screen is for a very definite purpose in absorbing certain parts of the light, it follows that the character of the light and that of the color screen must be mutually adapted. For example, it is self-evident that the same color screen for a given preparation would not serve for both daylight and the light from a mazda lamp (see fig. 45). So also the same color screen would not be successful if used both for the mazda light and for the light of a kerosene flame.

For the most successful use of color screens and different light sources, one should have curves of the intensity of the light in different parts of the visible spectrum like that for the mazda lamp and sunlight (fig. 45). Then one should know the absorption by each color filter for each kind of light. Knowing these facts and the absorbing and transmitting qualities of his specimens, and the sensitiveness of the photographic plates used, one could make intelligent selections and reasonably expect good results.

§ 501. **Exposure with color screens.** — The color screen naturally increases the time of exposure. It depends on the color and density of the screen. In general the exposure is increased from 2 to 5 times. The increase necessary is usually given by the manufacturers, therefore each individual worker does not have to find out by experiment. There is plenty of opportunity for the use of his judgment with the different qualities of his specimens (§ 497).

§ 502. **Developers.** — It is best to use the developers recommended by the manufacturers of the plates used. The experts employed by the manufacturers have found the best means for developing the plates, and it is safe to follow their advice. One usually has a choice of developers; and as a general statement it should be said that the beginner would be wise to prefer a slow developer, for it allows a greater latitude than a rapid developer. In general, a developer containing much bromide works slowly and gives very strong contrasts. Sometimes this is desirable, but often it is better to get the soft effects that come with a small amount of bromide. If one studies the little manuals sent out by the manufacturers, there will be found formulæ which give the various effects desired. (See collateral reading suggested at the end of the chapter.)

§ 503. **Light to develop by.** — The light which can be used in the dark room depends upon the sensitiveness of the plates or the printing paper used. The more sensitive the plates or paper, the less light. Furthermore, the sensitiveness to the different wave lengths is also important to consider. If the plates are sensitive only to the violet-blue of the spectrum, the dark room can be quite brightly lighted with red light with entire safety. If isochromatic or orthochromatic plates are used, they are sensitive to the spectrum up to and including yellow, and hence the dark-room light must exclude those, or be red only.

For panchromatic plates which are sensitive to all wave lengths the only safe method is to develop in total darkness, for any light will fog the plate if it acts sufficiently upon it. Sometimes very dark green is used, for the eye is most sensitive to green if the light is very dim, although for bright light the eye is most sensitive to yellow. But to be able to see clearly enough to determine the stage of development by the green light dim enough to be safe, one must be in the dark room for half an hour or more. The total darkness method is safest. One learns rather quickly to work in total darkness, and the time during which development goes on can be determined by counting seconds, or by a signal clock ringing minutes or by an alarm clock which can be set at the beginning for the estimated time to be used. Or finally, one can develop in a tray which is covered so that no light can reach the plate; then the ordinary dark-room light can be turned on from time to time to see when the estimated period for development has been reached.

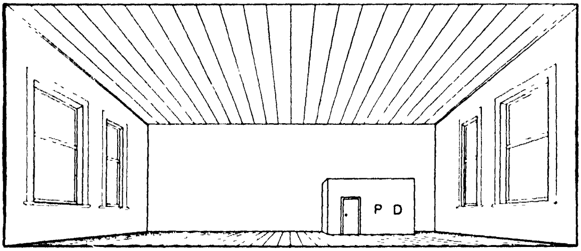


FIG. 214. DARK ROOM FOR PHOTOGRAPHY AND DRAWING IN A LARGE ROOM. (From Optic Projection).

It is far safer to use too little light for developing rather than too much. For ordinary or for isochromatic plates only a brief glance occasionally is all that is needed. If one holds the plate in the dark-room light during the whole development or for a considerable time there is almost always a thin veil of fog which lessens the crispness of the picture.

The wisdom of the advice to develop isochromatic or ordinary plates with as small an exposure to the dark-room light as possible can be demonstrated by the beginner in the following experiment which he is advised to try.

Put an isochromatic or orthochromatic plate in the plate holder. Pull out the dark slide till one or two centimeters of the film is exposed, then leave this for half a minute close to the developing-room light. Pull out the slide another centimeter or two and expose again to the dark-room light. Continue till the entire plate has been exposed. The last segment will have an exposure of half a minute, next to the last a whole minute, and so on. Now develop the picture in the ordinary way and the chances are that the plate will show very marked light effects, and the different segments in proportion to the time they were exposed to the dark-room light.

§ **504. Time development.** — Assuming that the correct plate and color screen are used, careful experiments made in the scientific laboratories of the large plate manufacturers have shown that the best method of developing photographic negatives is that of developing a definite time at a definite temperature of the developer. The time and temperature must, of course, be determined for the special plate and composition of developer to be used. The variable then is the exposure of the plate. A perfectly timed plate will contain all the desired detail in the shadows and just sufficient density in the high lights so that the print will be sufficiently white. The deepest shadows in such a negative will be almost perfectly transparent.

A convenient and safe method of developing plates by the time method without having the room absolutely dark and without exposing the plate to any harmful light, is the following: The dark-room safelight is directed away from the developing tray and a shield put in position to further screen it. An alarm or other large-faced clock, with second hand, is put close to the safelight. This light may then be very dim and still illuminate the clock face sufficiently. If using isochromatic or orthochromatic plates, the red safelight is good; but if panchromatic or spectrum plates are used, the green safelight is better. The exceedingly minute amount of light reaching the plate from the safelight as here recommended can cause no damage (Henry Phelps Gage, Optical Department, Corning Glass Works).

§ 505. Choice of plates and color screens. — The hints given in the little manuals sent out by the manufacturers on request by their patrons give excellent hints for the selection of plates and color screens for a wide variety of objects. The beginner cannot do better than to follow those suggestions faithfully, until his own experience enables him to supplement those suggestions. Finally, of course, one wishes to be able to use his own judgment.

In general, if any color is present in the object to be photographed, one will have better success with isochromatic or orthochromatic plates, which are sensitive to violet-blue, green, and yellow, than with the ordinary plates, which are sensitive only to the violet-blue of the spectrum (figs. 211–212). If the colors involved contain orange and red, the isochromatic plates are not adequate, and one must then use panchromatic or spectrum plates, sensitive to all wave lengths (fig. 213).

For the color screen to employ, remember that color screens are not of real use for ordinary plates sensitive only to violet and blue. For isochromatic plates yellow color screens are very helpful for reducing the excessive effect of the violet and blue (§ 492) or for cutting them out altogether in getting contrast effects (§ 493). The same is true for panchromatic plates, only here a wider range of color screens can be used to get any desired contrast or compensating effect.

COLOR PHOTOGRAPHY

§ 506. Photographs in natural colors. — This has been the aim of experts in photography ever since its first invention. Lately methods have been devised by which surprisingly true color photographs have been produced. These color pictures are better adapted to large objects than to those with fine details such as are observed with the microscope. Still, many objects are fairly well represented in photo-micrographs.

The author's experience in color photography has been limited to the "Autochrome Process" (colored starch grain process). The directions in the small manual sent out with the plates are very clear. Any one familiar with the ordinary photographic processes can succeed in color photography. It may be said in passing that the pictures taken by this process are transparencies and must be looked at as such to bring out the colors. Furthermore, as colors are truly rendered only in daylight or by artificial daylight, these transparencies must be illuminated by natural or artificial daylight for a true rendering of the color.

While these pictures cannot be used as negatives to give paper prints in colors, they can be used as colored pictures to get the proper negatives for printing by the three-color process, so that with a good autochrome transparency, colored pictures for books and magazines can be produced without any hand being taken in the process by an artist; and for many things the transparency gives a truth and delicacy in coloring not attainable by the artist's brush.

§ **507. Photography with ultra-violet radiation.** — As the finest details of structure are more clearly brought out by the shorter wave lengths, it has been hoped for a long time that it would be finally possible to utilize the ultra-violet rays in photography, if not in vision.

As shown in the chapter on the ultra-violet microscope, quartz or other ultra-violet transmitting substance must be used for the reflector, the condenser and the slip for supporting the specimen. If one is to make photographs by the shorter ultra-violet wave lengths the cover-slip, the objectives and the oculars must also be of ultra-violet transmitting material like quartz, corex, etc. These materials are expensive, and it requires a high degree of skill on the part of the operator to manage the source of radiation and, indeed, the entire instrument. In spite of the difficulties, the promise of a fuller understanding of structure has spurred men on, and good results have already been attained. Promise of still greater results is bringing out new means and methods constantly. It may be remarked in passing, that with the apochromatic objectives, good photographs may be taken with radiation of wave length as short as 365 mμ (.0365μ) (3650 Ä).

COLLATERAL READING FOR CHAPTER X

BECK, CONRAD. — The Microscope, 1938 ed , p. 197+.

DR. AUGUST KÖHLER. — Eine mikrophotographische Einrichtung für ultraviolettes Licht (275 mμ) und damit angestellte Untersuchungen organischer Gewebe. Physikalische Zeitschrift, 5 Jahrgang, pp. 666–673. Four text figures of apparatus.

—— Mikrophotographische Untersuchungen mit ultraviolettem Licht. Zeitschrift für wissenschaftliche Mikroskopie und fur mikroskopische Technik. Band XXI, 1904, pp. 129–165, und 273–304, six plates.

ERNST, HAROLD C., M. D. AND WOLBACH, S. B., M. D. — Ultra-Violet Photomicrography. The Journal of Medical Research, Vol. XIV, (N. S. vol. ix. No. 3) pp. 463–469, April, 1906, seven plates.

LUCAS, FRANCIS F. (Bell Telephone Laboratories). — The Architecture of Living Cells. A discussion of recent advances in methods of biological research by means of optical sectioning with the ultra-violet microscope. Proceedings of the National Academy of Sciences, Vol. 16, pp. 599–607, Sept. 1930. 6 plates, 5 text figures.

LUCAS AND STARK. — Jour. Morph., vol. 52, 1931, pp. 91–113. Many photo-micrographs by ultra-violet.

MARTIN, L. C. — Some recent developments in Microscopy. Journal of the Royal Society of Arts, Vol. 79, 1931, pp. 871–885; 887–896. Polarizing and Ultra-Violet Microscopes.

Optic Projection, by S. H. & H. P. Gage.
The Wratten Booklets on Photographic Plates and Color Filters.
The Photography of Colored Objects, by C. E. Kenneth Mees.
Photo-micrography. Published by the Eastman Kodak Co.
Seed Plates, formulæ and directions. Eastman Kodak Co.

Furnished by the G. Cramer Dry Plate Company:
Cramer's Manual on Negative Making and Formulas.
Isochromatic Landscape Photography.
The Photographing of Color Contrasts.
Dry Plates and Filters for Trichromatic Work.
Photo-micrographic and Spectrographic Color Filters.

These brochures are naturally very recent and give the meat of the information at present available on the kind of photographic plates available and the proper color filters to use with them to produce the best effects with different colored objects in gross photography and in photo-micrography.

For the sensitiveness of the human eye to the different parts of the spectrum see: Herbert E. Ives, Philosophical Magazine, Vol. XXIV, 6th ser. Dec. 1912, pp. 853–863; P. G. Nutting, Transaction of the Illuminating Engineering Society, 1914, pp. 633–642.

Photo-technique. — New York, 1940 +.

CHAPTER XI

CABINETS; SLIPS AND COVER-GLASSES; MOUNTING; LABELING AND STORING MICROSCOPIC PREPARATIONS; REAGENTS

§§ 508-615; FIGURES 215-249

§ 508. Slides, glass slides or slips, microscopic slides or slips. — These are strips of clear, flat glass quartz or corex upon which microscopic specimens are usually mounted for preservation and ready examination. The size that has been almost universally adopted for ordinary preparations is 25 × 76 millimeters (1 × 3 inches). For rock sections, slides 25 × 45 mm. or 32 × 32 mm. are used; for serial sections, slides 25 × 76 mm., 38 × 76 mm. or 50 × 76 mm. are used.

For the ultra-violet microscope the slips must be transparent to the ultra-violet radiation. Quartz is best. The Corex D. glass of the Corning Glass Works is also good and much less expensive (§ 308).

As these quartz and corex slips look like glass, it seems to the author that a different size should be used, therefore he has adopted that of 25 × 65 mm. (1 × 2.6 inches). It is also desirable to put the name on one end with a writing diamond (fig. 218).

For special purposes, glass slips of the necessary size are employed without regard to any conventional standard.

Thick slips are preferred by many to thin ones. They should correspond in thickness with the working distance of the condenser with which one works, especially if that is of the achromatic-aplanatic type. Dr. Chamot recommends that they be of half length for chemical work. He adds further: " It is a great misfortune that the colorless glass slips used in America and so excellent for ordinary microscopic work should be easily attacked by all liquids; even water extracts a relatively enormous amount of alkalies and alkaline earths. The slips of greenish glass, while not as neat or desirable for general microscopy, seem to be decidedly more resistant, and are therefore preferable." Transparent celluloid slides are recommended by Behrens for work where hydrofluoric acid and its derivatives

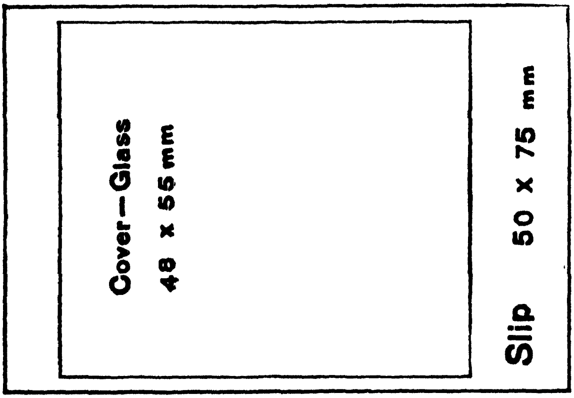


FIG. 215.

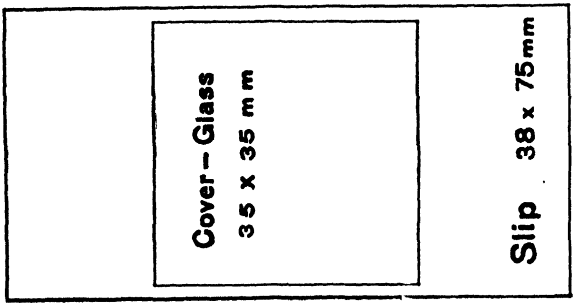


FIG. 216.

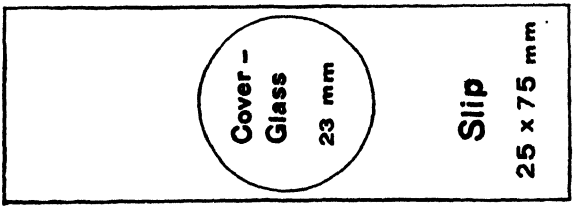


FIG. 217.

STANDARD SIZES OF GLASS SLIPS.

are to be examined. (Chamot, Jour. Appl. Micr., vol. iii, p. 793. Chemical Microscopy, pp. 123–124).

§ **509. Thickness of slips for special purposes.** — It is very important to observe strictly the requirements for the thickness of slip for special purposes. As pointed out in discussing the dark-ground condenser (§ 194), the slip must be thin enough so that the focus of the condenser will be just above the upper surface where the object is mounted. If the slip is too thick, the focus will be beneath the object and the best light cannot be obtained. So likewise with the best achromatic condensers, especially when used as homogeneous immersion condensers (§ 265), if the slip is too thick, the focus of the condenser will fall below the object and the best and most critical images cannot be obtained.

It is better to use a slip thinner than the maximum permissible, and plenty of homogeneous liquid between the slip and the condenser, then the condenser can be lowered until its focus is upon the object. This applies equally with the dark-ground condenser. For getting the thickness of the slips, use the micrometer calipers or a cover-glass measurer (figs. 219–220).

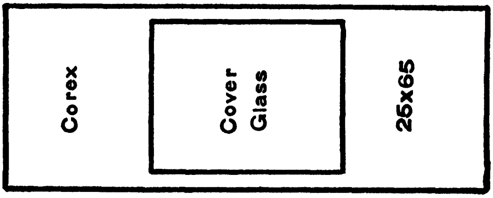


FIG. 218. COREX GLASS SLIP

25 × 65 mm.

These are transparent to ultra-violet, and should be used whenever the specimens are to be examined under the ultra-violet microscope.

§ **510. Cleaning slips for ordinary use.** — Place new slips that are to be wiped at once in a glass vessel of distilled water containing 5% ammonia. For wiping the slips use a lintless or a well-washed linen towel. One may avoid large wash bills by using absorbent gauze (§ 510a).

In handling the slips grasp them by the edges. Cover the fingers of the right hand with the wiping towel or the gauze and rub both faces with it. When the slide is wiped thoroughly dry, place it in a dry glass jar or for larger numbers use a museum jar (fig. 248). Soap and water are also recommended for new slips.

Alcohol of 50% to 82% is also excellent for cleaning new slips, and for those which have been freed from mounting media by boiling (§ 511) after a thorough rinsing in clean water.

§ **510a. Absorbent gauze and lintless towels.** — The gauze mentioned is No. 10, *sterilized absorbent gauze.* It is sometimes called bleached cheese cloth. In the author's laboratory it is cut into pieces, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ of a yard. When a piece is soiled, it is thrown away. There has recently appeared specially prepared towels for wiping glass, etc., which are called "lintless," as practically no lint is left on the wiped object. These are furnished by Johnson & Johnson of New York, and cost about 15 cents each in a size 42 × 90 cm.

§ **511. Cleaning used slips.** — If only watery substances or glycerin or glycerin jelly have been used, one may soak the slips overnight in ammonia water, then change the water for fresh and wipe as described in § 510.

When balsam or other resinous media (§ 564) have been used, it is best to heat the slips over a Bunsen flame and remove the cover-glass. Place the covers in cleaning mixture (§ 519). The slip may also be placed in cleaning mixture or in some hot water containing 10% gold dust or other strong alkaline cleaner. When the metal basin — preferably an agateware basin — is two-thirds full of the slips, heat until the water comes to a boil. Then let it cool. Add fresh water and most of the slips may be wiped clean.

If dichromate cleaning mixture is used, the best method is to have a museum jar of it and drop the slips in as they are rejected, or a large number at once, as is most convenient. It may require a week or more to clean the slips with cleaning mixture. As this is a very corrosive mixture for metals, use only glass dishes in dipping into it. When the slips are freed from balsam, etc., pour off the cleaning mixture into another glass vessel and allow a stream of water to flow over the slips until all the cleaning mixture has been washed away. Then add water and wipe the slips from that. Any slips still not freed from the balsam should be put back into the cleaning mixture.

§ **512. Cleaning slips for special uses.** — In making blood films, for micro-chemistry and whenever an even film is desired, every particle of oily substance must be removed, and every other foreign substance. In a word the glass must be made thoroughly clean. To accomplish this end the writer has found a slight modification of the method of Stitt the most effective and convenient. (See Stitt, " Practical Bacteriology, Blood Work and Animal Parasitology," 7th ed., 1923, p. 299). New slips or those that have been cleaned as described in § 511, are placed one by one into a glass or agateware dish containing an emulsion of bon ami. For the emulsion 5 grams of the bon ami powder is stirred up with 100 cc. of water. The slips are stirred around in this emulsion and then taken out one by one and set up on end on blotting paper or gauze to dry. When thoroughly dry, they are placed in a box for future use. Whenever a slip is needed, it is wiped well with a piece of fresh white gauze or one of the lintless towels. As remarked by Stitt, this is better than any other single method or all of the others combined.

The gauze mentioned is of the heavier grade, white and absorbent. It has been used several years in our laboratories, and has been found satisfactory and economical. For use a square yard is cut into 16 equal pieces for cleaning and polishing glass slips. For cover-glasses a square yard is cut into 64 equal pieces. In taking blood samples one of these small pieces should be used but once and then discarded.

The best way to tell when slips or cover-glasses are free from a surface film is to drop some water upon the glass and then incline it to a sloping position. If the glass surface is clean, the water will run over the glass and leave a wet track. If a film of oily substance is present, the water will crawl and form ridges or droplets and will not leave a smooth wet surface. Sometimes it is almost impossible to get a slip so that a film of blood or other substance can be spread evenly upon it. Probably the simplest thing in such a case is to use such a slip for mounting sections in balsam; but Chamot, pp. 149–150, says that they may in many cases be made suitable by passing them slowly through a Bunsen flame.

§ **513. Cover-glasses or covering glasses.** — These are circular or quadrangular pieces of thin glass used for covering and protecting microscopic objects. They should be very thin, 0.10 to 0.25 millimeter. It is better never to use a cover-glass over 0.20 mm. thick, then the preparation may be studied with a 2 mm. oil immersion as well as with lower objectives. Except for objects wholly unsuited for high powers, it is a great mistake to use cover-glasses thicker than the working distance of a homogeneous objective (§ 101). Indeed, if one wishes to employ high powers, the thicker the section the thinner should be the cover-glass.

The cover-glass should always be considerably larger than the object over which it is placed.

§ **514. Cleaning cover-glasses for ordinary use.** — Covers may be cleaned well by placing them in 82% or 95% alcohol containing hydrochloric acid one per cent. They may be wiped almost immediately.

Remove a cover from the alcohol, grasping by the edge with the left thumb and index. Cover the right thumb and index with some clean gauze or other absorbent cloth; grasp the cover between the thumb and index and rub the surfaces, keeping the thumb and index well opposed on directly opposite faces of the cover so that no strain will come on it, otherwise the cover is likely to be broken.

When a cover is dry hold it up and look through it toward some dark object. The cover will be seen partly by transmitted and partly by reflected light, and any cloudiness will be easily detected. If the cover does not look clear, breathe on the faces and wipe again. If it is not possible to get a cover clean in this way, it should be put again into the cleaning mixture or thrown away.

As the covers are wiped put them in a clean shell-vial (fig. 227), glass box or Petri dish. Handle them by their edges, or use fine forceps. Do not put the fingers on the faces of the covers, for that will surely cloud them.

§ **515. Cleaning cover-glasses for special uses.** — As with glass slips, cover-glasses intended for films or other purposes where the least particles of oily substance or other foreign material must be removed, are most satisfactorily cleaned by Stitt's bon ami method. New cover-glasses or cleaned used ones are put into a bon ami emulsion, 5 grams to 100 cc. of water, in a shallow dish like a saucer or plate and moved about somewhat.

They are then taken out one by one and set on edge against and on clean blotting paper or gauze exactly as for slips (§ 512); or, following Mt. Holyoke College, a tray is covered with clean gauze and the covers laid one by one upon it. The tray is inclined to about 40 degrees and when the excess liquid has run down to the lower edge of the covers, it is blotted off. When dry, the covers are stored in a glass box.

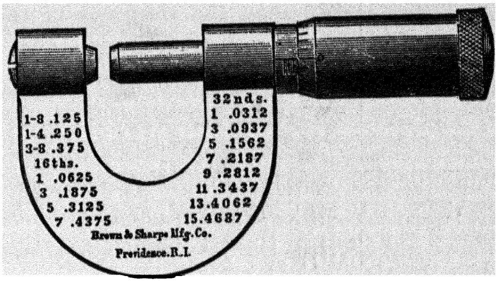


FIG. 219. BROWN & SHARPE'S MICROMETER CALIPERS.

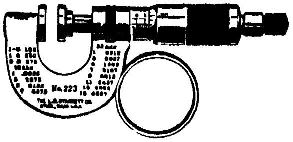


FIG. 220. STARRETT'S PAPER-GAUGE MICROMETER CALIPERS.

When ready to use a cover-glass, wipe it with one of the small pieces of gauze. If especially exacting, use a fresh piece of gauze for each cover.

Ordinarily many cover-glasses and slips are cleaned at one time and stored for future use. If the preparations are to be mounted in Canada balsam, this method answers fairly well, but it is not satisfactory in dark-field microscopy. Experience also shows that even when stored in glass receptacles, the cleaned covers and slips gradually accumulate a surface film which renders them unfit for even balsam mounts unless they are recleaned. The cleaning is so rapid and thorough by the Stitt bon ami method that even for mounting series it is not a great burden to wipe the slips and covers as they are needed.

§ 516. Cleaning large cover-glasses for serial sections. — These large, quadrangular covers are put one by one in bon ami emulsion and treated in every way like the glass slips and small cover-glasses.

§ 517. Measuring the thickness of cover-glasses. — It is desirable to know the thickness of the covers: for (*a*) in studying the preparation one would not try to use objectives of a shorter working distance than the thickness of the cover (§ 101); (*b*) in using adjustable objectives with the collar graduated for different thicknesses of cover, the collar can be set at a favorable point without loss of time; (*c*) for unadjustable objectives the thickness of cover may be selected corresponding to that for which the objective was corrected (§ 254). Furthermore, if there is a variation from the standard, one may remedy it, in part at least, by lengthening the tube if the cover is thinner, and shortening it if the cover is thicker than the standard (§ 256).

Among the so-called No. 1 cover-glasses of the dealers in microscopical supplies, the writer has found covers varying from 0.10 mm. to 0.35 mm. To use cover-glasses of so wide a variation in thickness without knowing whether one has a thick or thin one is simply to ignore the fundamental principles by which correct microscopic images are obtained.

From information supplied by Mr. Edward Pennock the thickness of various cover-glasses should be within the following limits:

No. 1 cover-glasses	0.12 to 0.18 mm.
No. 2	0.18 to 0.25 mm.
No. 3	0.25 to 0 50 mm.
No. 0	0.10 mm. slightly more or less.

In general cover-glasses thinner than the minimum (0.12 mm.) of No. 1, actual measurement, will, as stated above, usually show a much wider variation.

It is then strongly recommended that every preparation shall be covered with a cover-glass whose thickness is known, and that this thickness be indicated in some way on the label (fig. 234).

§ 518. Micrometer calipers for measuring glass slips and cover-glasses. — The micrometer gauges in figs. 219–220 are satisfactory for getting the thickness of slips and covers. The paper gauge (fig. 220) is a little safer for cover-glasses as they are grasped by a broader surface. These instruments may be had for the inch standard or for the millimeter standard.

With these measures or gauges one should be certain that the index stands at zero when at rest. If the index does not stand at zero, it should be adjusted at that point, otherwise the readings will not be correct.

As the covers are measured, the different thicknesses should be put into different glass boxes and properly labeled. Unless one is striving for the most accurate possible results, cover-glasses varying not more than 0.06 mm. may be put in the same box. For example, if one takes **0.15** mm. as a standard, covers varying 0.03 mm. on each side may be put into the same box. In this case the box would contain covers of 0.12, 0.13, 0.14, **0.15**, 0.16, 0.17, and 0.18 mm.

§ **519. Dichromate cleaning mixture for glass.** — The cleaning mixture used for cleaning slides and cover-glasses is that commonly used in chemical laboratories: (Dr. G. C. Caldwell's Laboratory Guide in Chemistry.)

Dichromate of potash ($K_2Cr_2O_7$)	200 grams
Water, distilled or ordinary (H_2O)	800 cc.
Sulphuric acid (H_2SO_4)	1200 cc.

As great heat is developed in the reaction on mixing the sulphuric acid with the watery solution of dichromate, it is necessary to use heat-resisting vessels. The best so far employed are those made of pyrex glass. Use ordinary tap water and the commercial dichromate and strong sulphuric acid. Chemically pure ingredients are not demanded.

Dissolve the dichromate in the water by the aid of heat. Use for this an agate dish. Now place the pyrex dish in the sink on some asbestos or a piece of board. Pour the warm solution of dichromate into the pyrex dish, and then add the sulphuric acid, stirring the liquid with a glass rod. The reaction is so great that the liquid will boil violently. An abundance of chromic acid crystals will form as the sulphuric acid is added. Let the pyrex dish remain in the sink until the cleaning mixture is cool and then pour it into a glass-stoppered bottle for storage.

If the dichromate is well pulverized, it can be put directly into the pyrex dish with the requisite amount of water, and the sulphuric acid added as directed.

This is an excellent cleaning mixture and is practically odorless. It is exceedingly corrosive and must be kept in glass vessels. It may be used more than once, but when the color changes markedly from that seen in the fresh mixture it should be thrown away. An indefinite sojourn of the slides and covers in the cleaner does not seem to injure them.

MOUNTING, AND PERMANENT PREPARATION OF MICROSCOPIC OBJECTS

§ **520. Mounting a microscopic object** is so arranging it upon some suitable support and in some suitable mounting medium that it may be satisfactorily studied with the microscope.

The cover-glass on a permanent preparation should always be considerably larger than the object; and where several objects are put under one cover-glass, as with serial sections, it may be confusing to crowd them too closely together.

§ **521. Temporary mounting; normal fluids.** — In a great many cases objects do not need to be preserved; they are then mounted in any way to enable one best to study them, and after the study the cover-glass is removed, and the slide cleaned for future use. In the study of living objects, of course only temporary preparations are possible. With amœbæ, white blood corpuscles, and many other objects, both animal and vegetable, the living phenomena can best be studied by mounting them in the natural medium. That is, for amœbæ, the water in which they are found; for the white blood corpuscles, a drop of blood is used and, as the blood soon coagulates, they are in the serum. Sometimes it is not easy or convenient to get the natural medium; then some liquid that has been found to serve in place of the natural medium is used. For many things, water with a little common salt (water 1000 cc., common salt, NaCl, 8 grams) is employed. This is the so-called isotonic or normal salt or saline solution. For the ciliated cells from frogs and other amphibia, nothing has been found so good as human spittle. Whatever is used, the object is put on the middle of the slide and a drop of the mounting medium added, and then the cover-glass.

The cover is best put on with fine forceps, as shown in fig. 221. After the cover is in place, if the preparation is to be studied for some time, it is better to avoid currents and evaporation by painting a ring of castor oil around the cover in such a way that part of the ring will be on the slide and part on the cover (fig. 235).

It cannot be too strongly emphasized that if one is to study living or fresh tissues, they must be mounted in a liquid which will not injure them. The liquid in which they are naturally found is of course the most nearly normal of any, and should be always used when possible. Water seems a very bland and harmless liquid, but it has a very decidedly injurious effect on living tissues which are normally bathed by the fluids of the body, for they always contain salts and colloid material. Distilled water is more deleterious than tap water because it contains no salts. It would be deleterious to water organisms, because all natural waters contain a greater or lesser quantity of organic and inorganic substances in solution. If the water supply of a city or town has a filtration plant, the water is likely to be unsuitable for raising water forms like salamander embryos, and the embryos of the frogs and toads, besides many other water forms. One must take the trouble to get the water from the natural breeding places if the embryos are to be successfully raised in a laboratory. (See also §§ 542–543, 606.)

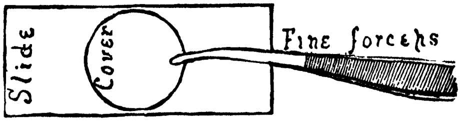


FIG. 221. FINE FORCEPS FOR HANDLING COVER-GLASSES AND OTHER DELICATE OBJECTS.

§ **522. Permanent mounting.** — There are three great methods of making permanent microscopic preparations. Special methods of procedure are necessary to mount objects successfully in each of these ways. The best mounting medium and the best method of mounting in a given case can be determined only by experiment. In most cases some previous observer has already made the necessary experiments and furnished the desired information.

The three methods are the following:

(1) Dry or in air (§§ 523–526).

(2) In some medium miscible with water, as glycerin or glycerin jelly (§§ 527–531).

(3) In some medium like Canada balsam, damar, petrolatum, etc. (§§ 532–536).

§ **523. Mounting dry or in air.** — The object should be thoroughly dry. If any moisture remains, it is likely to cloud the cover-glass, and the specimen may deteriorate. As the specimen must be sealed, it is necessary to prepare a cell slightly deeper than the object is thick. This is to support the cover-glass, and also to prevent the running in by capillarity of the sealing mixture.

Order of procedure in mounting objects dry or in air.

1. A cell of some kind is prepared. It should be slightly deeper than the object is thick (§ 525).
2. The object is thoroughly dried (desiccated) either in dry air or by the aid of gentle heat.
3. If practicable, the object is mounted on the cover-glass; if not, it is placed in the bottom of the cell.
4. The slide is warmed till the cement forming the cell wall is somewhat sticky, or a very thin coat of fresh cement is added; the cover is warmed and put on the cell and pressed down all around till a shining ring indicates its adherence.
5. The cover-glass is sealed.
6. The slide is labeled.
7. The preparation is catalogued and safely stored.

§ **524. Example of mounting dry, or in air.** — Prepare a shallow cell and dry it (§ 525). Select a clean cover-glass slightly larger than the cell. Pour upon the cover a drop of 10% solution of salicylic acid in 95% alcohol. Let it dry spontaneously. Warm the slide till the cement ring or cell is somewhat sticky; then warm the cover gently and put it on the cell, crystals down. Press on the cover all around the edge, seal, label and catalogue.

A preparation of mammalian red blood corpuscles may be made satisfactorily by spreading a very thin layer of fresh blood on a cover with the end of a slide. After it is dry, warm gently to remove the last traces of moisture and mount blood side down, precisely as for the crystals. One can get the blood as directed for the chylomicrons in dark-field work (§ 212).

§ **525. Preparation of mounting cells.** — (A) *Thin cells.* These are most conveniently made of some of the cements used in microscopy. Shellac is one of the best and most generally applicable. To prepare a shellac cell place the slide on a turn-table (fig. 222) and center it, that is, get the center of the slide over the center of the turn-table. Select a guide ring on the turn-table which is a little smaller than the cover-glass to be used, take the brush from the shellac, being sure that there is not enough cement adhering to it to drop. Whirl the turn-table and hold the brush lightly on the slide just over the guide ring selected. An even ring of cement should result. If it is uneven, the cement is too thick or too thin, or too much was on the brush. After a ring is thus prepared remove the slide and allow the cement to dry spontaneously, or heat the slide in some way. Before the slide is used for mounting, the cement should be so dry when it is cold that it does not dent when the finger nail is applied to it.

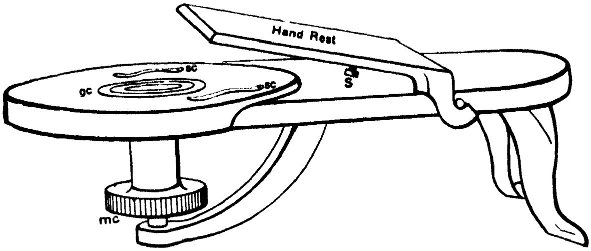


Fig. 222. Turn-table for Making Cells and for Sealing Cover-glasses.

Hand Rest The metal plate supporting the hand that holds the brush. It can be raised or lowered by means of the screw underneath (*s*).

sc Spring clips for holding the slide in place.

gc Guide circles to aid in centering the slide or the mounted object.

mc Milled circular disc by which the turn-table is whirled when the ring of cement is being painted around the cover-glass or the mounting cell.

A cell of considerable depth may be made with the shellac by adding successive layers as the previous one dries.

(B) *Deep cells* are sometimes made by building up cement cells, but more frequently, paper, wax, glass, hard rubber or some metal is used for the main part of the cell. Paper rings, block tin or lead rings are easily cut out with gun punches. These rings are fastened to the slide by using some cement like the shellac.

(C) Cells for square and oblong covers can be made freehand.

§ **526. Sealing the cover-glass for dry objects mounted in cells.**— When the cover is in contact with the wall of cement all around (§ 523), the slide should be placed on the turn-table and arranged so that the cover-glass and cell wall will be concentric with the guide rings of the turn-table. Then the turn-table is whirled and a ring of fresh cement is painted, half on the cover and half on the cell wall (fig. 235). If the cover-glass is not in contact with the cell wall at any point and the cell is shallow, there will be great danger of the fresh cement running into the cell and injuring or spoiling the preparation. When the cover-glass is properly sealed, the preparation is put in a safe place for the drying of the cement. It is advisable to add a fresh coat of cement occasionally. Seal the square and oblong covers freehand.

§ **527. Mounting objects in media miscible with water.** — Many objects are so greatly modified by drying that they must be mounted in some medium other than air. In some cases water with something in solution is used. Glycerin of various strengths and glycerin jelly are also much employed. All these media keep the object moist and therefore in a condition resembling the natural one. The object is usually and properly treated with gradually increasing strengths of glycerin or fixed by some fixing agent before being permanently mounted in strong glycerin or either of the other media.

In all of these different methods, unless glycerin of increasing strengths has been used to prepare the tissue, the fixing agent is washed away with water before the object is finally and permanently mounted in either of the media.

§ **528. Order of procedure in mounting objects in glycerin.** —

1. A cell must be prepared on the slide if the object is of considerable thickness (§ 525).

2. A suitably prepared object is placed on the center of a clean slide, and if no cell is required a centering card is used to facilitate the centering (fig. 223).

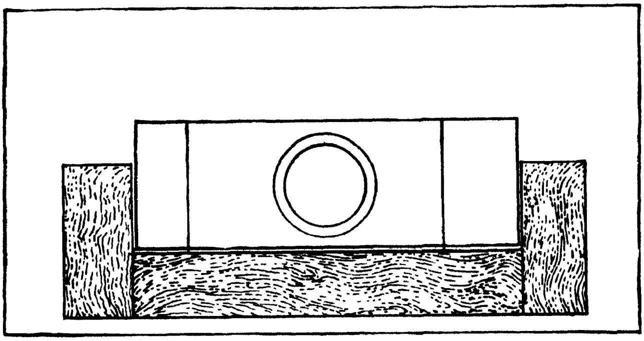


FIG. 223. GUIDE CARD TO AID IN MOUNTING OBJECTS NEATLY.

3. A drop of pure glycerin is poured upon the object, or if a cell is used, enough to fill the cell and a little more.

4. In putting on the cover-glass it is grasped with fine forceps and the underside breathed on to moisten it slightly so that the glycerin will adhere; then one edge of the cover is put on the cell or slide and the cover gradually lowered upon the object. The cover is then gently pressed down. If a cell is used, a fresh coat of cement is added before mounting.

5. The cover-glass is sealed.

6. The slide is labeled.

7. The preparation is catalogued and safely stored.

§ **529. Order of procedure in mounting objects in glycerin jelly.** —

1. Unless the object is quite thick, no cell is necessary with glycerin jelly.

2. A slide is gently warmed and placed on the centering card (fig. 223) and a drop of warmed glycerin jelly is put on its center. The suitably prepared object is arranged in the center of the slide.

3. A drop of the warm glycerin jelly is then put on the object, or if a cell is used, it is filled with the medium.

4. The cover-glass is grasped with fine forceps, the lower side breathed on and then gradually lowered upon the object and gently pressed down.

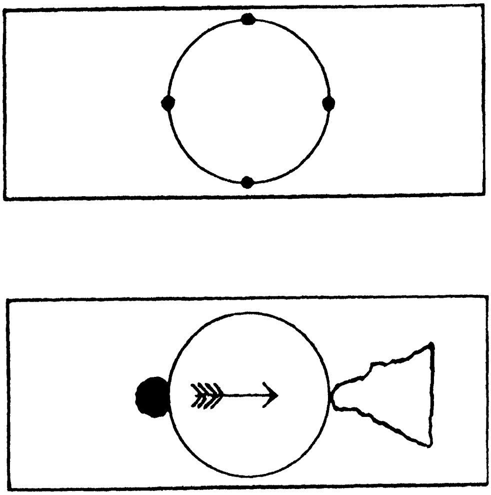


Fig. 224. Corex Glass Slips 25 × 65 mm.

The upper one shows the method of anchoring the cover-glass by means of four drops of shellac. The lower one shows the method of irrigating a preparation. A drop of the solution is put on one side of the cover and a piece of blotting paper on the opposite side. The arrow shows the direction of the flow toward the blotting paper. As the irrigating liquid will be strongest or most abundant in the middle, all stages of its action on the preparation may be seen on the sides.

5. After mounting, the preparation is left flat in some cool place till the glycerin jelly sets; then the superfluous amount is scraped and wiped away and the cover-glass sealed with shellac (§ 530).

6. The slide is labeled.

7. The preparation is catalogued and safely stored.

§ 530. Sealing the cover-glass when no cell is used. — (A) *For glycerin-mounted specimens.* The superfluous glycerin is wiped away as carefully as possible with a moist cloth; then four minute drops of cement are placed at the edge of the cover (fig. 224) and allowed to harden for half an hour or more. These will anchor the cover-glass so that it can be sealed.

(B) *For objects in glycerin jelly, Farrants' solution or a resinous medium.* The mounting medium us first allowed to harden; then the superfluous medium is scraped away as much as possible with a knife, and then removed with a cloth moistened with water for the glycerin jelly and Farrants' solution; or with alcohol, chloroform or turpentine, etc., if a resinous medium is used. Then the slide is put on a turn-table and a ring of the shellac cement is added.

§ 531. Example of mounting in glycerin jelly. — For this select some stained and isolated muscular fibers or other suitably prepared objects (§§ 537–541). Arrange them on the middle of a slide, using the centering card, and mount in glycerin jelly as directed in § 529. Air bubbles are not easily removed from glycerin jelly perparations, so care should be taken to avoid them.

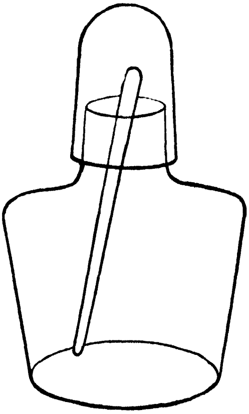


FIG. 225. SMALL SPIRIT LAMP USED AS A CONTAINER FOR GLYCERIN, BALSAM, ETC.

§ 532. Mounting objects in resinous media. — While the media miscible with water offer many advantages for mounting animal and vegetable tissues, the preparations so made are likely to deteriorate. In many cases, also, they do not produce sufficient transparency to enable one to use high enough powers for the demonstration of minute details.

By using sufficient care almost any tissue may be mounted in a resinous medium and retain all its details of structure.

For the successful mounting of an object in a resinous medium it must in some way be deprived of all water and all liquids not miscible with the resinous mounting medium. There are two methods of bringing this about: (A) by drying or desiccation (§ 533), and (B) by successive displacements (§ 535).

§ 533. Order of procedure in mounting objects in resinous media by desiccation:

1. The object suitable for the purpose (fly's wings, etc.) is thoroughly dried in dry air or by gentle heat.

2. The object is arranged as desired in the center of a clean slide on the centering card (fig. 223).

3. A drop of the mounting medium is put directly upon the object or spread on a cover-glass.

4. The cover-glass is put on the specimen with fine forceps (fig. 221), but in no case does one breathe on the cover as when media miscible with water are used.

5. The cover-glass is pressed down gently.

6. The slide is labeled.

7. The preparation is catalogued and safely stored (§ 548).

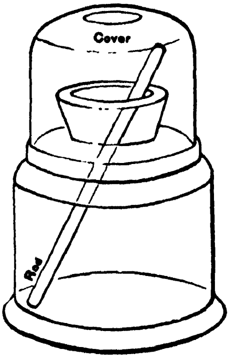


Fig. 226. Container for Canada Balsam, Glycerin Jelly, etc.

Cover The glass cover to keep out dust and prevent evaporation.

Rod The glass rod for transferring the contents of the container to the slide.

§ 534. Example of mounting in balsam by desiccation. — Find a fresh fly, or, if in winter, procure a dead one from a window sill or a spider's web. Remove the fly's wings, being especially careful to keep them the dorsal side up. With a camel's hair brush remove any dirt that may be clinging to them. Place a clean slide on the centering card, then with fine forceps put the two wings within one of the guide rings. Leave one dorsal side up, turn the other ventral side up. Spread some Canada balsam on the face of the cover-glass and with the fine forceps place the cover upon the wings (fig. 221). Probably some air-bubbles will appear in the preparation, but if the slide is put in a warm place these will soon disappear. Label, catalogue, etc.

§ 535. Mounting in resinous media by a series of displacements. — For examples of this see the procedure in the paraffin and in the collodion methods, Ch. XII. The first step in the series in *dehyrdation;* that is, the water is displaced by some liquid which is miscible with both the water and the next liquid to be used. Strong alcohol (95% or stronger) is usually employed for this. Plenty of it must be used to displace the last trace of water. The tissue may be soaked in a dish of the alcohol, or alcohol from a pipette may be poured upon it. Dehydration usually occurs in the thin objects to be mounted in balsam in 5 to 15 minutes. If a dish of alcohol is used, it must not be used too many times, as it loses in strength.

The second step is clearing. That is, some liquid which is miscible with the alcohol and also with the resinous medium is used. This liquid is highly refractive in most cases, and consequently this step is called *clearing* and the liquid a *clearer.* The clearer displaces the alcohol, and renders the object more or less translucent. In case the water was not all removed, a cloudiness will appear in parts or over the whole of the preparation. In this case the preparation must be returned to alcohol to complete the dehydration.

One can tell when a specimen is properly cleared by holding it over some dark object. If it is cleared, it can be seen only with difficulty, as but little light is reflected from it. If it is held toward the window, however, it will appear translucent.

The third and final step is the displacement of the clearer by the resinous mounting medium.

The specimen is drained of clearer and allowed to stand for a short time till there appears the first sign of dullness from evaporation of the clearer from the surface. Then a drop of the resinous medium is put on the object, and finally a cover-glass is placed over it, or a drop of the mounting medium is spread on the cover and it is then put on the object. For abundant examples see the next chapter.

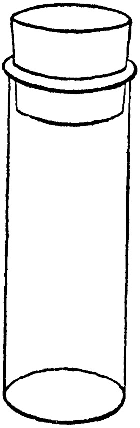
§ 536. Mounting in petrolatum liquidum, pure mineral oil. — As this substance does not fluoresce, and is of nearly the refractive index of glass it serves well for mounting unstained sections for the ultra-microscope and also as an immersion liquid.

The unstained sections are freed from the solid paraffin as usual (§ 638) and the oil added. No clearing is necessary. It is then covered, and the cover-glass sealed with shellac or with ambroid or other pyroxylin cement.

ISOLATION OF HISTOLOGIC ELEMENTS

§ 537. Isolation, general. — For a correct conception of the forms of the cells and fibers of the various organs of the body, one must see these elements isolated and thus be able to inspect them from all sides. It frequently occurs also that the isolation is not quite complete, and one can see in the clearest manner the relations of the cells or fibers to one another.

The chemical agents or solutions for isolating are, in general, the same as those used for hardening and fixing. But the solutions are only about one-tenth as strong as for fixing, and the action is very much shorter, that is, from one or two hours to as many days. In the weak solution the cell cement or connective tissue is softened so that the cells and fibers may be separated from one another, and at the same time the cells are preserved. In fixing and hardening, on the other hand, the cell cement, like the other parts of the tissue, is made firmer. In preparing the isolating solutions it is better to dilute the fixing agents with normal salt solution than merely with water (§ 606).



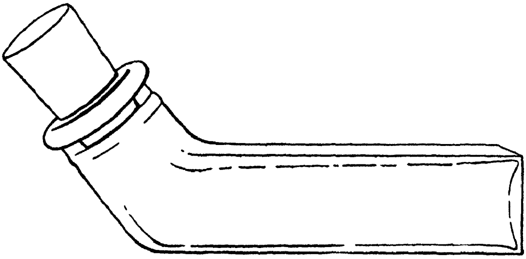


FIG. 227, 228. SHELL VIAL AND COMSTOCK, BENT-NECK SPECIMEN BOTTLE.

Shell vial with turned lip. One can have almost any size and length desired. Those of 22 × 65 mm. and 30 × 90 mm. have been found most useful. The larger ones are excellent for staining single slides or pairs.

The Comstock, bent-neck specimen bottle is very useful for keeping small animals straight.

§ 538. Isolation by means of formaldehyde. — Formaldehyde in normal salt solution is one of the very best dissociating agents for brain tissue and all the forms of epithelium. It is prepared as follows: 2 cc. of strong formalin (that is, a 40% solution of formaldehyde) are mixed with 1000 cc. of normal salt solution. This acts quickly and preserves delicate structures like the cilia of ordinary epithelia and also of the endymal cells of the brain. It is satisfactory for isolating the nerve cells of the brain. For the epithelium of the trachea, intestines, etc., the action is sufficient in half an hour; good preparations may also be obtained any time within two days or more. The action on nerve tissue of the brain and myel or spinal cord is about as rapid.

§ **539. Staining the cells.** — Almost any stain may be used for the formalin dissociated cells. For example, one may use eosin. This may be drawn under the cover of the already mounted preparation (fig. 224), or a new preparation may be made and the scrapings mixed with a drop of eosin before putting on the coverglass. It is an advantage to study unstained preparations, otherwise one might obtain the erroneous opinion that the structure cannot be seen unless it is stained. The stain makes the structural features somewhat plainer; it also accentuates some features and does not affect others so markedly. Congo red is excellent for most isolated cells.

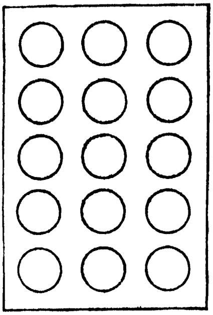


FIG. 229. BLOCK WITH HOLES FOR SHELL VIALS.

The blocks are about 33 mm. thick and the holes are bored clear through, then a board about 5 mm. thick is nailed on the bottom.

§ **540. Permanent preparations of isolated cells.** — If one desires to make a permanent preparation of isolated cells it may be done by placing a drop of glycerin at the edge of the cover and allowing it to diffuse under the cover, or the diffusion may be hurried by using a piece of blotting paper, as shown in fig. 224. One may also make a new preparation by mixing thoroughly some of the isolated material with congo-glycerin. After a few minutes the cover-glass may be put on and sealed (§ 530). If one adds congo-glycerin to a considerable amount of the isolated material it may be kept and used at any time.

§ 541. Isolation of muscular fibers. — For this the formalin dissociator may be used (§ 538), but the nitric acid method is more successful (§ 581). The fresh muscle is placed in this in a glass vessel. At the ordinary temperature of a sitting room (20 degrees centigrade) the connective tissue will be so far gelatinized in from one to three days that it is easy to separate the fascicles and fibers either with needles or by shaking in a test-tube or shell vial with water. It takes longer for some muscles to dissociate than others, even at the same temperature, so one must try occasionally to see if the action is sufficient. When it is, the acid is poured off and the muscles washed gently with water to remove the acid. If one is ready to make the preparations at once, they may be isolated and mounted in water. If it is desired to keep the specimen indefinitely or several days, the water should be poured off and 2% formaldehyde added. The specimens may be mounted in glycerin, glycerin jelly or balsam. Glycerin jelly is the most satisfactory, however.

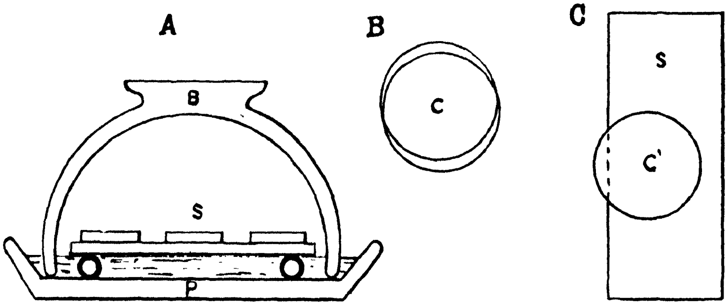


FIG. 230. MOIST CHAMBER AND MOIST PREPARATIONS.

A Bowl (*B*) inverted over a plate (*P*) containing water and a glass shelf supported on glass rods. The slides (*S*) are supported on the glass shelf. This makes a very efficient and cheap moist chamber.

B Cover-glasses (*C*) made slightly eccentric and containing between them the object to be kept moist. By using cover-glasses the specimen can be examined from both sides, and as part usually remains with each cover-glass, two permanent preparations can be made.

C Slide (*S*) with a cover-glass (*C*) extending slightly over one edge so that it can be lifted up without danger of sliding it along and thus disarranging the specimen.

COLLECTION AND STUDY OF MICROSCOPIC ANIMALS AND PLANTS

§ 542. Collection of material. — There are many microscopic forms in nature that need no other preparation than mounting on a glass slide. If low powers are used, a cover-glass may be omitted, but if high powers are to be used, a cover-glass must be put over the object to protect the objective as well as the object, and to make the optical corrections of the objective perfect (§ 254).

The easiest place to find things most interesting and beautiful is in the water of pools and along the shores of streams where the water is quiet. Go to some pond or stream and along the shore where it is shallow; take some of the vegetation and the mud, put in a pail or dish, and take to the home or laboratory. Put the water and vegetation in a plate or other shallow vessel and put it in about the same light that it had in nature. In a few hours, when the mud has settled the conditions will be nearly as in nature, and by the use of fine forceps or one of the pipettes (figs. 221–231), gather some of the water with scrapings from some of the vegetation, or some of the water and mud. Put it on a slide, cover and examine. There may be much to see or very little. One must persevere and finally there will come a kind of instinctive knowledge where to find things. It is also a good plan to use the tripod or other magnifier and examine the dish. Often much can be seen in that way, and one will get a hint where to collect

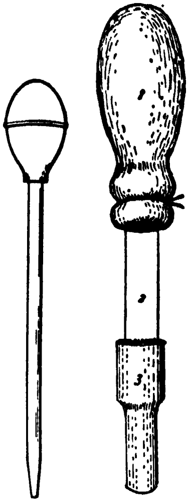


FIG. 231. PIPETTES FOR LIQUIDS AND FOR SPECIMENS.

A Pipette for liquids. This is about one-third size.

B Pipette for handling ova and other delicate specimens.

1 The rubber bulb tied to the glass part. It is about natural size.

2 Glass rod. The upper end is fluted so that the rubber bulb will not come off, and the lower end is carefully smoothed by heating. To prevent small ova and other objects getting into the bulb, some fine gauze may be tied over the upper end.

3 Soft rubber tube over the lower end. This is not absolutely necessary, but the soft rubber is less likely to injure delicate objects than the hard glass.

the bits to put on the slide for examination. Do not use distilled water for these organisms, but water from the source of supply. (For food see § 543.)

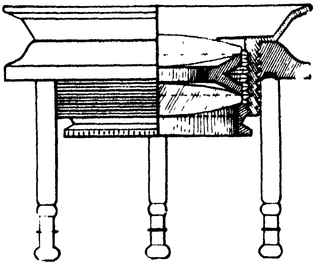


FIG. 232. TRIPOD MAGNIFIER.

§ **543. Infusoria and bacteria; Infusions.** — One of the best ways to get a large variety of living forms, animal and vegetable, is to make such a gathering as described above and to put it into a small fruit jar or other wide open vessel, and to put with it some of the stems of the grass along the stream. If in a moderately warm place for a day or more, this collection will be found swarming with living things. Soon, however, the numbers will lessen and finally there will be very few left. These living things need food. One of the good foods for them is made from boiling up some of the grass and hay found near the natural habitat. Any good hay may be used, however. When the mixture is cool, add some of it to the vessel containing the organisms, or what is better, take another dish, and add a fair amount of the liquid from the first gathering. Usually this new supply will be as rich in life as was the original gathering. (See under Neutral Red (§ 604) for experiment in staining live forms).

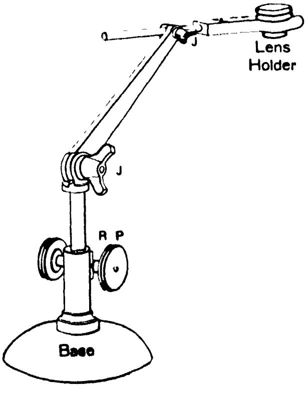


FIG. 233. MAGNIFIER SUPPORTED BY A FOCUSING, JOINTED HOLDER.

Base The heavy iron base to keep the apparatus steady.

R P Rack and pinion for focusing the magnifier.

J J Joints to make it possible to put the lens in any desired position.

§ **544. Diatoms.** — These are plants with silicious shells, and are found in natural waters both salt and fresh. If one goes to a pond or stream in May or June or July especially, the diatoms are very abundant. They may be found at any time, but in the spring most abundantly, as with most living things. The brownish or rusty looking substance on plants, rocks, etc., practically always contains diatoms, and sometimes is made up mostly of them. It is most interesting to study the diatoms alive and watch them glide around in the water. The shells of the diatoms have been favorite objects of study for a long time. They are often beautifully marked. Being silicious, they resist acids, and the living substance in and around them can be destroyed without hurting the shells. This may be done by placing the material containing a large number of diatoms in a test-tube. When the diatoms have settled, pour off a part of the liquid or draw it out with the pipette (fig. 231 *A*), and add an equal amount of nitric acid. Boil for a few minutes, let the diatoms settle, pour or draw off most of the liquid, and add more nitric acid and boil again. Finally, add water and gradually wash the diatom shells by drawing off the water and adding fresh. The shells should be clean and almost colorless and show their markings well. One can take a sample and see if the cleaning is sufficient. (For full and elaborate directions see Boyer's Diatomaceæ of Philadelphia and Vicinity, pp. 122–123).

§ **545. Arranging minute objects.** — Minute objects like diatoms or the scales of insects may be arranged in geometrical figures or in some fanciful way, either for ornament or for more satisfactory study. To do this the cover-glass is placed over the guide. This guide for geometrical figures may be a net-micrometer or a series of concentric circles. In order that the objects may remain in place, however, they must be fastened to the cover-glass. As an adhesive substance, mucilage or liquid gelatin, this thinned with an equal volume of 50% acetic acid answers well. A very thin coating of this is spread on the cover with a needle, or in some other way, and allowed to dry. The objects are then placed on the gelatinized side of the cover and carefully put into position with a mechanical finger, made by fastening a cat's whisker in a needle holder. For most of these objects a simple microscope with stand (figs. 232–233) will be found of great advantage. After the objects are arranged, one breathes very gently on the cover-glass to soften the mucilage or gelatin. It is then allowed to dry, and if a suitable amount of gelatin has been used and it has been properly moistened, the objects will be found firmly anchored. In mounting one may use Canada balsam or mount dry in a cell (§§ 526, 533). See Newcomer, Amer. Micr. Soc.'s Proc., 1886, p. 128; see also E. H. Griffith and H. L. Smith, Amer. Jour. of Micros., iv, 102, v, 87; Amer. Monthly Micr. Jour., i, 66, 107, 113; Cunningham, The Microscope, viii, 1888, p. 237.

LABELING, CATALOGUING AND STORING MICROSCOPIC PREPARATIONS

§ **546.** Every person possessing a microscopic preparation is interested in its proper management; but it is especially to the teacher and investigator that the labeling, cataloguing and storing of microscopic preparations are of importance. "To the investigator, his specimens are the most precious of his possessions, for they contain the facts which he tries to interpret, and they remain the same while his knowledge, and hence his power of interpretation, increase. They thus form the basis of further or more correct knowledge; but in order to be safe guides for the student, teacher, or investigator, it seems to the writer that every preparation should possess two things: viz., a label and a catalogue or history. This catalogue should indicate all that is known of a specimen at the time of its preparation, and all of the processes by which it is treated. It is only by the possession of such a complete knowledge of the entire history of a preparation that one is able to judge with certainty of the comparative excellence of methods, and thus to discard or improve those which are defective. The teacher, as well as the investigator, should have this information in an accessible form, so that not only he, but his students, can obtain at any time all necessary information concerning the preparations which serve him as illustrations and them as examples."

§ **547. Labeling ordinary microscopic preparations.** — The label should possess at least the following information:

The number of the preparation, its name and date and the thickness of the sections and of the cover-glass.

§ **548. Cataloguing preparations.** — It is believed from personal experience, and from the experience of others, that each preparation (each slide or each series) should be accompanied by a catalogue containing at least the information suggested in the following formula. This formula is very flexible, so that the order may be changed, and numbers not applicable in a given case may be omitted. With many objects, especially embryos and small animals, the time of fixing and hardening may be months and even years earlier than the time of imbedding. So, too, an object may be sectioned a long time after it was imbedded, and finally the sections may not be mounted at the time they are cut. It would be well in such cases to give the date of fixing under 2, and under 5, 6 and 8, the dates at which the operations were performed, if they differ from the original date and from one another. In brief, the more that is known about a preparation, the greater its value.

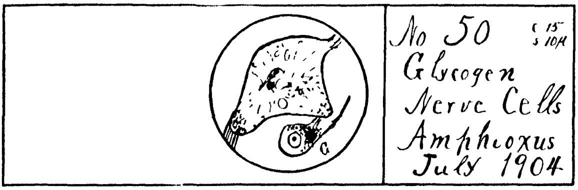


FIG. 234. LABEL FOR A MICROSCOPIC PREPARATION.

The specimen is the myel (spinal cord) of an Amphioxus showing the dorsal and ventral nerve roots, and some nerve cells near the middle.

G A nerve-cell with glycogen.

In the label *c.15* means that the cover-glass is 0.15 mm. in thickness; and *s. 10 μ* means that the section is ten microns thick. The date at the bottom shows when the specimen was made.

§ **549. General formula for cataloguing microscopic preparations:**

1. The general name and source. Thickness of cover-glass and of section.

2. The number of the preparation and the date of obtaining and fixing the specimen; the name of the preparator.

3. The special name of the preparation and the common and scientific name of the object from which it is derived. Purpose of the preparation.

4. The age and condition of the object from which the preparation is derived. Condition of rest or activity; fasting or full fed at the time of death.

5. The chemical treatment, — the method of fixing, hardening, dissociating, etc., and the time required.

6. The mechanical treatment, — imbedded, sectioned, dissected with needles, etc. Date at which done.

7. The staining agent or agents and the time required for staining.

8. Dehydrating and clearing agent, mounting medium, cement used for sealing.

9. The objectives and other accessories (micro-spectroscope, polarizer etc.), for studying the preparation.

10. Remarks, including references to original papers, or to good figures and descriptions in books.

§ 550. A catalogue card written according to this formula:

1. Muscular Fibers of Cat; Cover 0.15 mm.; Fibers 20μ to 40μ thick.

2. No. 475. (Drr. IX) Oct. 1, 1891. S. H. G., Preparator.

3. Tendinous and intra-muscular terminations of striated muscular fibers from the *Sartorius* of the cat (*Felis domestica*).

4. Cat eight months old, healthy and well nourished. Fasting and quiet for 12 hours.

5. Muscle pinned on cork with vaselined pins and placed in 20 per cent nitric acid immediately after death by chloroform. Left 36 hours in the acid; temperature 20° C. In alum water ($\frac{1}{2}$ sat. aq. sol.) 1 day.

6. Fibers separated on the slide with needles, Oct. 3.

7. Stained 5 minutes with Delafield's hematoxylin.

8. Dehydrated with 95% alcohol 5 minutes, cleared 5 minutes with carbol-turpentine, mounted in xylene balsam; sealed with shellac.

9. Use a 16 mm. for the general appearance of the fibers, then a 2 or 3 mm. objective for the details of structure. Try the micro-polariscope.

10. The nuclei or muscle corpuscles are very large and numerous;

many of the intra-muscular ends are branched. See S. P. Gage, Proc. Amer. Micr. Soc., 1890, p. 132; Ref. Hand-book Med. Sci., Vol. V, p. 59.

§ **551. General remarks on catalogues and labels.** — It is especially desirable that labels and catalogues shall be written with some imperishable ink. Some form of waterproof carbon ink is the most available and satisfactory. The waterproof ink of Higgins or Weber answers well. For ordinary writing it should be diluted with one-third its volume of water and a few drops of strong ammonia added.

If one has a writing diamond, it is a good plan to write a label with it on one end of the slide. It is best to have the paper label also, as it can be more easily read.

The author has found stiff cards, 12½ × 7½ cm., like those used for cataloguing books in public libraries, the most desirable form of catalogue. A specimen that is for any cause discarded has its catalogue card destroyed or stored apart from the regular catalogue. New cards may then be added in alphabetical order as the preparations are made. In fact a catalogue on cards has all the flexibility and advantage of the slip system of notes.

Some workers prefer a book catalogue. Very excellent book catalogues have been devised by Alling and by Ward (Jour. Roy. Micr. Soc., 1887, pp. 173, 348; Amer. Monthly Micr. Jour., 1890, p. 91; Amer. Micr. Soc. Proc., 1887, p. 233).

The fourth division has been added, as there is coming to be a strong belief, practically amounting to a certainty, that there is a different structural appearance in many if not all of the tissue elements, depending upon the age of the animal, upon its condition of rest or fatigue; and for the cells of the digestive organs, whether the animal is fasting or full fed. Indeed as *physiological histology* is recognized as the only true histology, there will be an effort to determine exact data concerning the animal from which the tissues are derived. (See Minot, Proc. Amer. Assoc. Adv. Science, 1890, pp. 271–289; Hodge, on nerve cells in rest and fatigue, Jour. Morph., vol. VII (1892), pp. 95–168; Jour. Physiol., vol. XVII, pp. 129–134; Gage, The Processes of Life revealed by the Microscope; a Plea for

Physiological Histology, Proc. Amer. Micr. Soc., vol. XVII (1895), pp. 3–29; Science, vol. II, Aug. 23, 1895, pp. 209–218. Smithsonian Institution, Report for 1896, pp. 381–396.

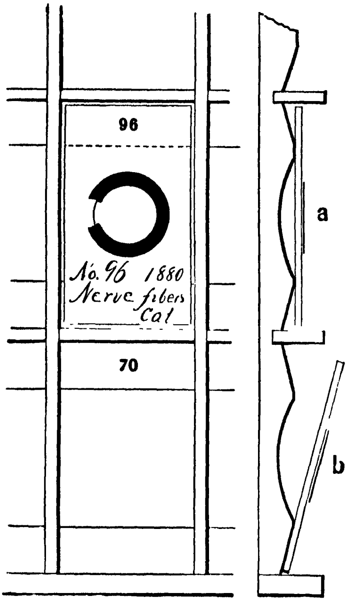


Fig. 235. Face and Edge View of a Cabinet Drawer for Microscopic Slides.

96, *70* The number of the compartment.

a b In the compartment *a*, the slide is resting in place to show that the container touches the slide only in two places.

In *b*, the slide is depressed into the groove at one end of the compartment. It is then easy to grasp the slide.

Cabinet for Microscopic Preparations

§ 552. While it is desirable that microscopic preparations should be properly labeled and catalogued, it is equally important that they should be protected from injury. During the last few years several forms of cabinets or slide holders have been devised. Some are very cheap and convenient where one has but a few slides. For a laboratory or for a private collection where the slides are numerous, the following characters seem to the writer essential:

(1) The cabinet should allow the slides to lie flat, and exclude dust and light.

(2) Each slide or pair of slides should be in a separate compartment. At each end of the compartment should be a groove or bevel, so that upon depressing either end of the slide, the other may be grasped easily (fig. 235). It is also desirable to have the floor of the compartment grooved so that the slide rests only on two edges, thus preventing soiling the slide opposite the object.

(3) Each compartment or each space sufficient to contain one slide of the standard size should be numbered, preferably at each end.

If the compartments are made of sufficient width to receive two slides, then the double slides so frequently used in mounting serial sections may be put into the cabinet in any place desired.

(4) The drawers of the cabinet should be entirely independent, so that any drawer may be partly or wholly removed without disturbing any of the others.

(5) On the front of each drawer should be the number of the drawer in Roman numerals, and the number of the first and last compartment in the drawer in Arabic numerals (fig. 236).

§ **553. Trays for slides and ribbons of sections.** — Early in 1897 the writer devised the simple tray shown in fig. 237. It was designed especially for the ribbons of sections in preparing embryologic series and for material in class work. As will be seen by the figure, the two sides are alike and the tray is very shallow. It was soon found that the wood forming the bottom of the tray was too rough for ribbons of sections and smooth white paper was put in the tray before the ribbons were laid upon it.

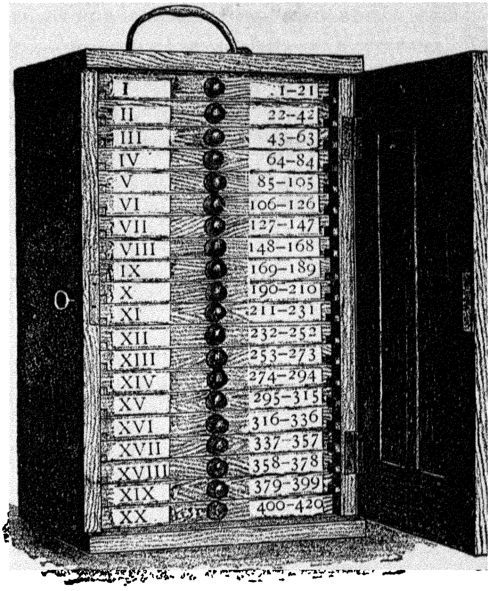


FIG. 236. CABINET FOR MICROSCOPE SLIDES.

This cabinet contains 20 drawers like that shown in fig. 235, and as indicated at the right there are 420 compartments for slides.

These trays were soon used for the mounted preparations as well as for the ribbons of sections. They were made of a proper size to fit the laboratory lockers (fig. 242) and naturally came to be used for storage instead of the expensive slide cabinets. For this purpose five could be put in a single compartment of the locker or thirty-five in an entire locker. As each tray holds fifty slides 25 × 75 mm., thirty-five 38 × 75 mm., and twenty-five slides 50 × 75 mm., the saving of space was very great.

§ **554. Slide trays with tongue groove, and compartments.** — In the first trays the edges were square and sharp. These were rounded in later trays, but there still remained a defect, for if one wished to pile up five to twenty trays on the table, they would not stay in an even stack. To remedy this defect the long way of the frame was tongued on one side and grooved on the other, as shown in fig. 238. This is a great improvement, as one can make even stacks of 25 or 50 trays, and they will stay in position. Furthermore it renders the groups of five trays stored in the locker compartments much easier to manage, as one can remove any of the five trays without getting

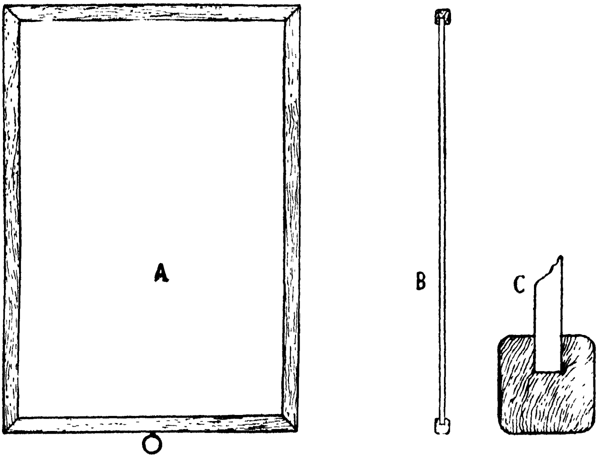


FIG. 237. SIMPLEST FORM OF SLIDE TRAY.

A Face view of the slide tray. The screw eye at the lower end is convenient for pulling out a single tray.

B Sectional view of the tray showing the thin board of which it is made and the wooden frame.

C Sectional view showing how the frame is fastened to the board.

the others disarranged, as so often occurred with the old form, lacking tongue and groove.

A defect of the trays for storage is the ease with which the slides get disarranged unless the tray is entirely full. To overcome this defect S. P. Gage divided one face of the tray into columns (fig. 238) by means of stout cord held in place by using melted paraffin as a cement. Later Dr. Greenman of the Wistar Institute divided one face of the tray into columns by wooden strips. This is the best way. With the tray face in columns the slides in a single column may become disarranged, but there is no mixing of the slides of different columns. One side of the tray remains smooth and can be used for ribbons of sections or for any other purpose. Dr. Jean Broadhurst

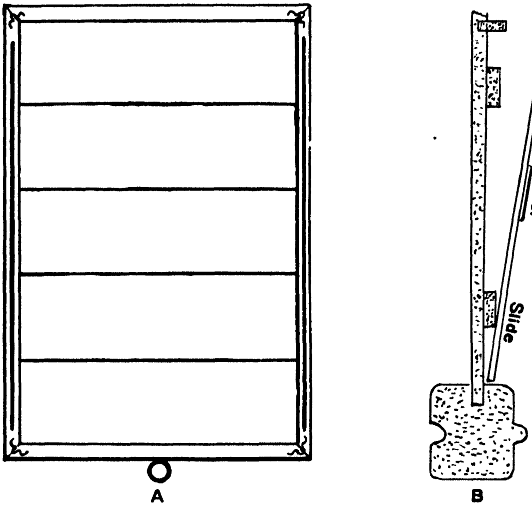


FIG. 238. SLIDE TRAY WITH COMPARTMENTS, TONGUE AND GROOVE.

A Face view of the new form of slide tray. It is designed for fifty slides, 25 × 75 mm. in size or twenty-five slides, 50 × 75 mm. in size used for serial sections of embryos, etc.

B Sectional view of a side piece with tongue and groove. Supports 5 mm. wide near the end prevent the slide from contact with the tray. This is especially important in dark-field or other work when oil-immersion liquid is on the lower face of the slide. The support also enables one to depress one end of the slide thus rendering it more easily grasped. See also figures 87, 235.

of Teachers College, Columbia University, has found that these trays are admirably suited for a cabinet of lantern slides. The smooth side will hold 13, two rows arranged lengthwise and one row crosswise. If a sheet of white paper is put under the slides, it is easy to see what is on them.

§ **554a.** The original maker of these trays was the H. J. Bool Co., of Ithaca, N. Y.; there are no restrictions, however, and excellent trays of the tongue, groove and column type (fig. 238 *A*) are now also available at the Clay-Adams Co.

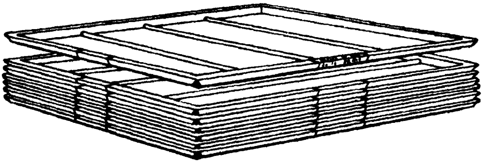


FIG. 239. THE WISTAR INSTITUTE METAL TRAY FOR MICROSCOPIC PREPARATIONS.

The upper tray was raised up and supported by corks when the photograph of the pile of trays was made. The picture shows the form, the rows for slides, and the band on the edge for writing labels.

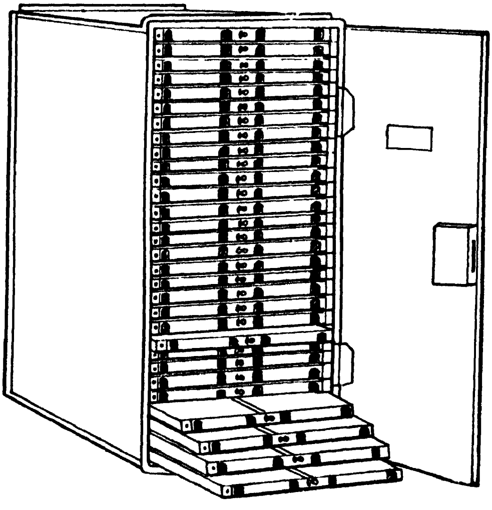


FIG. 240. THE MINOT METAL CABINET AND METAL TRAYS FOR MICROSCOPIC SLIDES.

(Courtesy of Peter Gray & Sons, Inc. East Cambridge, Mass.)

§ **555.** Metal slide trays of the Wistar Institute (fig. 239); Minot metal slide trays and cabinets (fig. 240); the Eberbach aluminum slide trays and cabinet (fig. 241); and the Paragon C. & C. Company Paragon cabinets for filing large numbers of slides in very small space.

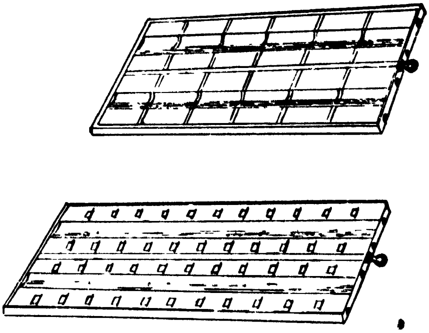


FIG. 240, A. B. TWO FORMS OF METAL TRAYS FOR THE MINOT SLIDE CABINET

A is for Slides 50 × 75 mm. or Two Standard Slides in Each Small Compartment.

Tray *B* is for standard size slides. The little metal tongues serve to make a place for standard size slides (25 × 75 mm.). In both trays there is a groove in the middle to facilitate lifting up the slides when needed. As shown also there is a little knob for pulling out the trays, and metal clips to hold paper labels.

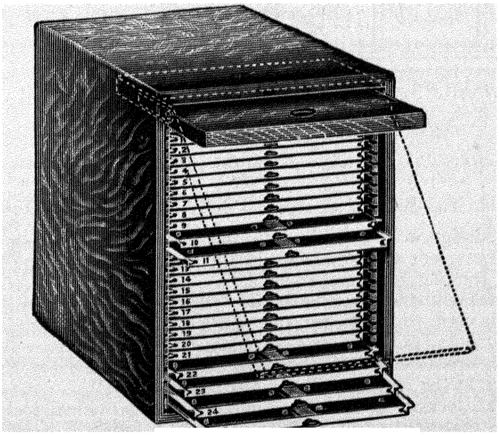


FIG. 241. THE EBERBACH ALUMINUM SLIDE TRAYS AND CABINET.
(Courtesy of Eberbach & Son Co.)

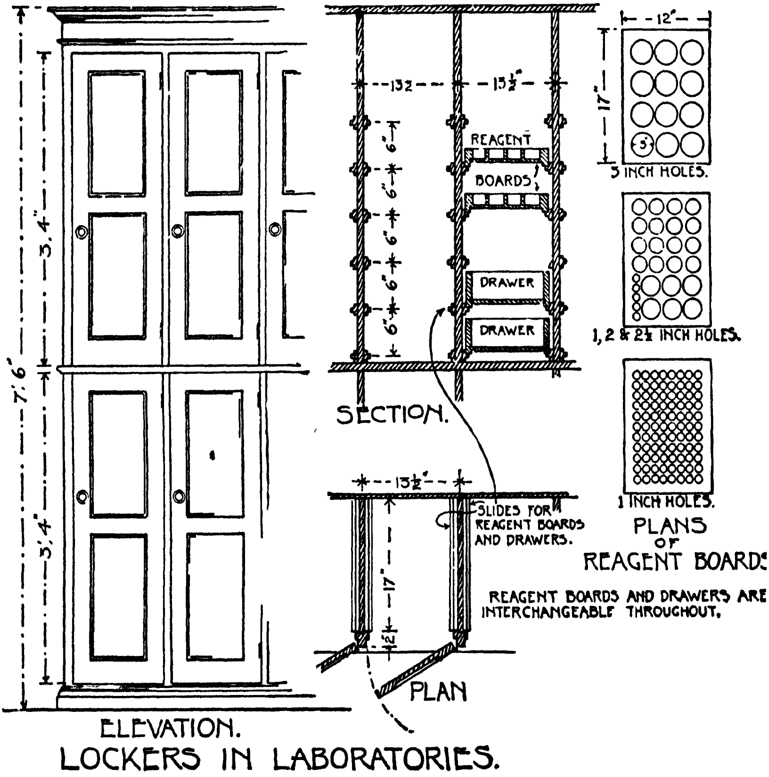


Fig. 242. Laboratory Lockers Reagent Boards and Drawers Designed in 1895.
(From the Journal of Applied Microscopy, 1898, p. 127).

The lockers designed in 1899 for Stimson Hall are in banks of 12 or 9, with three vertical tiers, not two as shown in this figure. Everything is of standard size and hence completely interchangeable.

Measured over all, the locker banks are 329 cm. high, and 139.5 cm. wide for the large banks and 105 wide for the smaller banks. Each individual locker, inside measure, is 32 cm. wide, 70.5 cm. high, and 48 cm. deep. It is divided by 7 runs into 8 compartments. As indicated in the sectional view, the entire space may be left free in the locker or partly filled or wholly filled.

Each bank of lockers is lettered, and then the individual lockers numbered from 1–12 or 1–9, the numbering being in the order of words in a book, i.e., from left to right. Of course vertical numbering is equally feasible. With this form of numbering each bank is practically independent and can be changed in position without confusion.

REAGENTS FOR MICROSCOPIC WORK

For much of the work done with a microscope the reagents needed are few and inexpensive, but for a large laboratory with the diversity of investigations carried on the reagents are numerous, and some of them expensive. Below are given some of the principal ones with the method of their preparation.

General on preparation of reagents. — In preparing reagents both weights and measures are used. As a rule the amounts given are those which experience has shown to give good results. Variations in the proportions of the mixtures are sometimes advantageous, and in almost every case a slight change in the proportions makes no difference. Most laboratory reagents are like food, good even under quite diverse proportions and methods of preparation. With a few, however, it is necessary to have definite strengths.

By a *saturated solution* is meant one in which the liquid has dissolved all that it can of the substance added. This varies with the temperature. It is well to have an excess of the substance present; then the liquid will be saturated at all temperatures usually found in the laboratory.

§ **556. Solutions less than 10 per cent.** — In making solutions where dry substance is added to a liquid, if the percentage is not over 10%, the custom is to take 100 cc. of the liquid and add to it the number of grams indicated by the per cent. That is, for a 5% solution one would take 100 cc. of the liquid and 5 grams of the dry substance. This does not make a strictly 5% solution. For that one should take 95 cc. of liquid and 5 grams of the dry substance; or, if the percentage must be exact, then one should weigh out 95 grams of the liquid and add 5 grams of the dry substance.

§ **557. Solutions of 10 per cent and more.** — When the percentage is 10% or over it is better to weigh out the number of grams representing the percentage and add to it the right amount of liquid in cubic centimeters. For example, if one were to make a 35% aqueous solution of caustic potash in water, then one would add 35 grams of caustic potash to 65 cc. of water. If one wished to make a 10% alcoholic solution of caustic potash, he would add 10 grams of caustic potash to 90 cc. of alcohol. But here is a case where, the alcohol being of less specific gravity than water, the mixture would not weigh 100 grams; and to make the mixture weigh 100 grams, giving therefore an exact percentage, one should take 90 grams of alcohol and add to it 10 grams of caustic potash. In practice in making solutions of collodion or parlodion one usually mixes ether and 95% or absolute alcohol in equal volumes and then for a 10% solution adds 10 grams of the dry soluble cotton or parlodion to 90 cc. of the ether-alcohol mixture. But ether is much lighter than water and the alcohol somewhat lighter, so that the percentage in this case would be more than 10% because the 90 cc. of alcohol and ether would weigh considerably less than 90 grams.

§ 558. **Mixtures of liquids to obtain a desired percentage.** — It frequently happens that it is desired to obtain a lower percentage or strength of a liquid than the one in stock. This is very readily done according to the general formula: Divide the percentage of the strong solution by the percentage of the desired solution and the quotient will show how many times too strong the stock solution is.

To get the desired strength, use 1 volume of the strong stock solution, and add to it enough of the diluting liquid to make a volume corresponding to the amount indicated by the quotient obtained by dividing the percentage of the stock solution by that of the desired solution. For example, if it is desired to obtain a 5% solution of formaldehyde from a stock solution of 40% strength, the stock solution being 8 times too strong, to get the 5% solution 1 volume of the strong solution must be used and 7 volumes of the diluting liquid (water). The solution so obtained will be $\frac{1}{8}$ of the original strength, or 5%.

If a 2% solution were desired then 1 volume of the strong solution would be taken and 19 volumes of water, etc.

§ 559. **Mixtures of alcohol.** — For alcohol if one desires a 50% solution it is usually near enough correct to add equal parts of 95% alcohol and water, but this does not actually give a 50% solution. To find the real proportions according to the general formula: 95% ÷ 50% = 1.9, i.e., for every 1 cc. of 95% alcohol should be added 0.9 cc. of water or for each 100 cc. of 95% alcohol, 90 cc. of water.

Even this will not give an exact mixture of alcohol, for a mixture of alcohol and water diminishes somewhat in volume. To get true percentages an alcoholometer for testing the specific gravity is used.

A simple method of getting approximately correct mixtures of alcohol is the following: Pour the strong alcohol into a graduate glass (fig. 243, *A*, *B*) until the volume is the same as the desired percentage; then add water until the volume is the same as the original percentage of the alcohol. Example: To get 50% from 95% alcohol put 50 cc. of 95% into a graduate and fill the graduate to 95 cc. with water, and the resulting mixture will be 50% alcohol, and so with all other strengths. Here the shrinkage is eliminated from consideration, because the water and alcohol are not measured separately and then mixed, but one is added to the other until a given volume is attained.

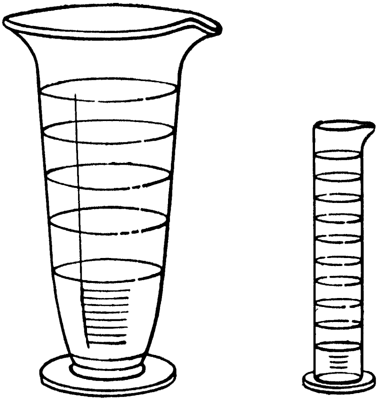


FIG. 243. GLASS GRADUATES FOR MEASURING LIQUIDS.

A Graduate with sloping sides for large quantities.

B Graduate with straight sides for smaller quantities and more accurate determination.

PREPARATION OF REAGENTS

§ **560. Albumen fixative (Mayer's).** — This consists of equal parts of well-beaten white of egg and glycerin. To each 50 cc. of this 1 gram of salicylate of soda is added to prevent putrefactive changes. Filter through absorbent cotton. It is not to be used on slides for the ultra-violet or incineration.

§ **561. Alcohol (ethyl), C_2H_5OH.** — Ethyl or grain alcohol is mostly used for histologic purposes. (A) Absolute alcohol (i.e., alcohol of 99%) is recommended for many purposes, but if plenty of 95% alcohol is used it answers every purpose in histology, in a dry climate or in a warm, dry room. When it is damp, dehydration is greatly facilitated by the use of absolute alcohol.

(B) 82% alcohol made by mixing 5 parts of 95% alcohol with 1 part of water.

(C) 67% alcohol made by mixing 2 parts of 95% alcohol with 1 part of water. See also §§ 558–559.

For educational and other public institutions the U. S. government grants the privilege of using ethyl alcohol without paying the revenue tax, but for private institutions and for individuals it would be a great relief if the denatured alcohol could be mixed in all proportions with water without the formation of precipitates.

§ **562. Alcohol (methyl), CH_3OH.** — Methyl alcohol or wood alcohol is much cheaper than ethyl or grain alcohol on account of the revenue tax on ethyl alcohol. It answers well for many microscopic purposes. It has been refined so carefully in recent years that the disagreeable odor is not very noticeable.

FIG. 244. GLASS-STOPPERED BOTTLES FOR THE MORE USUAL GRADES OF ALCOHOL USED IN MICROSCOPY.

Denatured alcohol. — This is ethyl or grain alcohol rendered undrinkable by the addition of wood alcohol and benzine (grain alcohol 89½%; methyl alcohol 10%, and benzine ½%). In some cases the denaturing substances are somewhat different, but all render the alcohol unusable for drinking. It is then free from internal revenue tax.

In Great Britain "*methylated spirits*" consists of grain alcohol with 10% methyl alcohol. This is used very largely in microscopic work. In America the addition of the benzine renders denatured alcohol also unfit for histological purposes if it is to be diluted. The addition of water makes it milky. If methyl alcohol alone or combined with pyridin or some other substance wholly soluble in water were used as the denaturing substance, denatured alcohol could be used in microscopic work for all the grades. That denatured as indicated above can be used only in full strength or very slightly diluted.

§ **563. Alcohol, normal propyl or propanol** ($CH_3CH_2CH_2OH$. — This form of alcohol has been shown by Sheridan to be especially useful in histology to prevent the great shrinking and hardening of tissues in imbedding by the paraffin method as it is a solvent of paraffin. (See Jour. Tech. Methods, and Bullt. Internatl. Assoc. Med. Museums, Vol XII, pp. 125–126, 1929 (Abstract in Stain Tech., Vol. V, 1930, p. 34. See § 641 for this method of use.

§ **564. Balsam, Canada balsam, balsam of fir.** — This is one of the oldest and most satisfactory of the resinous media used for mounting microscopic preparations.

The natural balsam is most often used; it has the advantage of being able to take up a small amount of water so that, if sections are not quite dehydrated, they will clear up after a time.

§ **565. Xylene balsam.** — This is Canada balsam diluted or thinned with xylene. It is recommended by many to evaporate the natural balsam to dryness and then to dissolve it in xylene. For some purposes, e.g., for mounting glycogen preparations, this is advantageous; but it is unnecessary for most purposes. Xylene balsam requires a very complete desiccation or dehydration of objects to be mounted in it, for the xylene is immiscible with water.

The hydrocarbon, xylene (C_8H_{10}) is called xylol in German. In English, members of the hydrocarbon series have the termination "ene," while members of the alcohol series terminate in "ol."

§ **566. Filtering balsam.** — Balsam is now furnished already filtered through filter paper. If xylene balsam is used, it may be made thin and filtered without heat. For filtering balsam and all resinous and gummy materials, the writer has found a paper funnel the most satisfactory. It can be used once and then thrown away. Such a funnel may be easily made by rolling a sheet of thick writing paper in the form of a cone and cementing the paper where it overlaps, or winding a string several times around the lower part. Such a funnel is best used in one of the rings for holding funnels, so common in chemical laboratories. The filtering is most successfully done in a very warm place, like an incubator or an incubator room.

§ **567. Artificial resins.** — There have been developed recently certain resin-like mounting media which promise to be even more satisfactory than Canada balsam. Two of them are: Du Pont's isobutyl methacrylate polymer and the Nevellite Co.'s clarite (cycloparaffin or naphthene polymer).

These when dissolved in xylene or toluene give a clear mounting medium which is of approximately the refractive index of glass and serve to give the stained sections the desired transparency.

Both plastics have been used for some months for mounting specimens to show glycogen stained with iodine. The stain is preserved perfectly.

For the *clarite* 60 grams in 40 cc. of xylene or toluene gives a solution of about the right consistency. The Du Pont methacrylate requires about equal percentage of the dry powder and the xylene or toluene. The clarite fluoresces somewhat in ultra-violet light and therefore should not be used for fluorescence mounts, § 602.

§ **568. Acid balsam.** — As stated above, all balsam is naturally somewhat acid, but for various stains it is desirable to increase the acidity. For example, specimens stained with picro-fuchsin, or injected with carmine or Berlin blue are more satisfactory and last longer with full brilliancy if the balsam is made more acid than it naturally is. For this use 10 to 20 drops of glacial acetic or formic acid to 100 cc. of balsam.

§ **569. Borax carmine for *in toto* staining.** — Borax 4 grams; carmine 3 grams; water 100 cc. Shake frequently for several days and then filter and add 100 cc. of 67% alcohol. After 3 to 4 days it may be necessary to filter again. Good for *in toto* staining after almost any fixer. Put the object to be stained from alcohol into a vial with plenty of stain. After a day or two change the stain. Stain 4 to 5 days. Remove to 67% alcohol, adding 4 drops of HCl to each 100 cc. of alcohol. After one day remove to 82% alcohol.

§ **570. Carmine for mucus (mucicarmin).** — One can buy the dry powder or preferably prepare the stain. To prepare it, take 1 gram of Carmine No. 40 and $\frac{1}{2}$ gram of pure dry ammonium chlorid. If the latter is slightly moist, dry it in an evaporating dish in a sand bath. Mix the ammonium chlorid and the carmine and add 2 cc. of water. Mix well and heat over a sand bath, constantly mixing with a glass rod. Continue the heating until the carmine colored mass becomes very dark red. It will take 3 to 10 minutes for this. The heat should not be too great.

Dissolve the dark red mixture in 100 cc. of 50% alcohol. For use, dilute five- or tenfold with tap water. This stains best after mercuric fixers. One must not collodionize sections to be stained with this, as the carmine stains the collodion very deeply. Stain the sections first with hematoxylin as usual; then stain 1 to 5 hours or longer with the dilute mucicarmin. The mucus in goblet cells, in the mucous part of the salivary glands, etc., will be red. Nuclei will be stained with hematoxylin. Mount in balsam (§ 535).

§ **571. Cedar-wood oil.** — For penetrating tissues and preparing them for infiltration with paraffin, thick oil is recommended by Lee. For tissues fixed with osmic acid for fat, the thick oil is necessary, but for most histologic and embryologic work, that known as Cedar-wood Oil (Florida) is excellent, also that known as Cedar-wood Oil (true Lebanon). These forms are far less expensive than the thick oil. The tissues should be thoroughly dehydrated before putting them into cedar-wood oil, and they should remain until they are translucent.

The thickened cedar-wood oil used for homogeneous immersion should be obtained from the manufacturers of microscopes; they naturally would supply the kind suitable for the purpose.

§ **572. Chloroform ($CHCl_3$).** — This is used for clearing and imbedding where fats fixed with osmic acid are to be preserved in the tissues. It is also used for hardening collodion, in collodion imbedding. It is an excellent solvent of cedar-wood oil and is used for cleaning homogeneous immersion fluid (cedar-oil) from objectives, condensers and microscopic preparations.

§ **573. Carbol-xylene clearer.** — Vasale recommends as a clearer, xylene 75 cc., carbolic acid (melted crystals) 25 cc.

§ **573a.** Carbol-xylene and eosin. In order to counterstain with eosin during the clearing process, the carbol-xylene is charged with eosin as follows: A saturated aqueous solution of eosin is prepared, and to it is added with constant stirring, hydrochloric acid until there is a good precipitate. Filter through filter paper. Wash the precipitate with distilled water until the water goes through pink. This indicates that the acid is washed out. Dry the washed precipitate. This is soluble in the carbol-xylene and enough should be added to make that pink. More or less can be used depending on the depth of the eosin stain desired. That can be regulated also by the time the sections are left in the eosined clearer. (Freeborn, Jour. Ap. Microscopy, Vol. III, p. 1058).

§ **574. Carbol-turpentine clearer.** — A satisfactory and generally applicable clearer is carbol turpentine, made by mixing carbolic acid crystals (*Acidum carbolicum. A. phenicum crystallizatum*) 40 cc. with rectified oil of turpentine (*Oleum terebinthinae rectificatium*) 60 cc. If the carbolic acid does not dissolve in the turpentine, increase the turpentine, thus: carbolic acid 30 cc., turpentine 70 cc.

This clearer is not so good as the preceding for mounting objects which have been stained with osmic acid, as the hydrogen dioxid (H_2O_2) present fades the blackened osmic acid.

§ **575. Clarifier, castor-xylene clarifier.** — This is composed of castor oil 1 part and xylene 3 parts. (Trans. Amer. Micr. Soc., 1895, p. 361.) For the use of this clarifier, see under the collodion method (§ 652).

§ **576. Collodion.** — This is a solution of soluble cotton or other form of pyroxylin in equal parts of sulphuric ether in 95% or absolute alcohol. In using soluble cotton for infiltrating and imbedding tissues several different strengths are used, commencing with weak and proceeding to strong mixtures. The last in which the tissue is imbedded is as thick a solution as can be made. All collodion solutions should be kept well corked, for the ether and alcohol are very volatile.

§ **576a.** The substance used in preparing collodion goes by various names; soluble cotton or collodion cotton is perhaps best. This is cellulose nitrate, and consists of a mixture of cellulose tetranitrate $C_{12}H_{16}(NO_3)_4O_6$ and cellulose pentanitrate, $C_{12}H_{15}(NO_3)_5O_5$. Besides the names soluble and collodion cotton, it is called gun cotton and pyroxylin. Pyroxylin is the more general term and includes several of the cellulose nitrates. Celloidin is a patent preparation of pyroxylin, more expensive than soluble cotton.

An American product known as "parlodion" has recently (1915) appeared to take the place of the celloidin not now obtainable. It is non-explosive, and is said to be a very pure, concentrated form of collodion especially adapted to the needs of histology and embryology. (Advertising pages, Anatomical Record, Dec., 1915.)

Soluble cotton should be kept in the dark to avoid decomposition. After it is in solution, this decomposition is not so likely to occur. The decomposition of the dry cotton gives rise to nitrous acid, and hence it is best to keep it in a box loosely covered, so that the nitrous acid may escape.

Cellulose nitrate is explosive under concussion and under 150° centigrade heat. In the air, the loose soluble cotton burns without explosion. It is said not to injure the hand if held upon it during ignition and not to fire gunpowder if burned upon it. So far as known to the writer, no accident has ever occurred from the use of soluble cotton for microscopic purposes. I wish to express my thanks to Professor W. R. Orndorff, organic chemist in Cornell University, for the above information. (Proc. Amer. Micr. Soc., vol. XVII (1895), pp. 361–370.)

§ **577. Collodion for cementing sections to the slide.** — This is a $\frac{3}{4}$% solution made by adding $\frac{3}{4}$ gram of soluble cotton to 50 cc. of 95% or absolute alcohol and 50 cc. of sulphuric ether. This may be used before deparaffining or, preferably, afterward. (See § 639.)

§ **578. Congo red.** — Water 100 cc., congo red $\frac{1}{2}$ gram. This is a good counter stain for hematoxylin.

§ **579. Congo-glycerin.** — (Glycerin 100 cc., congo red (§ 578) 20 cc.). For mixing with and staining isolation preparations (§ 539) and for a mounting medium, this is an excellent combination. It is particularly good for nerve cells.

§ **580. Decalcifier.** — One of the best is a mixture of ethyl alcohol and nitric acid (67% alcohol, 100 cc., strong nitric acid, 3 cc.). The tissue or organ should first be fixed by some approved method. It may then be put into the decalcifier. Change the decalcifier two or three times. It usually takes from 2 to 10 days, depending on the object and its size. One can tell when the decalcification is complete by inserting a fine needle. If there is no gritty feeling the decalcification is complete. Wash in two or three changes of water, and then transfer to 67% alcohol, and in 24 hours to 82% alcohol. It is better to imbed and section soon, as decalcified tissue is likely to deteriorate. The original fixation and the use of alcohol in the decalcifier are to avoid the gelatinization of the white fibrous tissue.

One can use either the collodion or the paraffin method for sectioning. For large objects perhaps the collodion method gives the better results.

§ **581. Dissociating Liquids.** — These liquids are for perserving the tissue elements or cells and for dissolving or softening the intercellular substance so that the cells may be readily separated from their neighbors. The separation is accomplished by (*a*) teasing with needles; (*b*) shaking in a liquid in a test-tube; (*c*) scraping with a scalpel and crushing with the flat of the blade; (*d*) tapping sharply on the cover-glass after the object is mounted. One may find it desirable to use (*d*) with all the methods.

(1) Formaldehyde dissociator. — Strong formalin (40% formaldehyde gas in water) 2 cc.; normal salt solution 1000 cc. One can begin work within ½ hour and good results may be obtained after 2 to 3 days immersion. Excellent for epithelia and for nerve cells.

(2) Müller's fluid dissociator. — Müller's fluid 1 cc., normal salt solution 9 cc. It usually requires from 1 to 5 days for epithelia to dissociate in this. The action is more rapid in a warm place.

(3) Nitric acid dissociator. — Nitric acid 20 cc., water 80 cc. This is used especially for muscular tissue. It takes from 1 to 3 days, depending on the temperature. The nitric acid gelatinizes the connective tissue. Wash out the acid with water for a few minutes. Preserve in 2% formaldehyde.

§ **582. Elastic stains.** — There are four good differential stains for elastic tissue:

(1) *The orcein stain of Unna* (Mon. Schr. Dermat., 1894, xix, 1). It is prepared by mixing 1 gram of orcein, 100 cc. of 82% alcohol, and 1 cc. of hydrochloric acid. Stain for one hour or less in the solution warmed in an oven (fig. 255). Wash well with 67% alcohol, then in water. Dehydrate and mount in balsam or clarite. Counterstain with hæmatoxylin or methylene blue. Elastic fibers stain dark brown, nuclei purple or blue (§§ 585, 593).

(2) *Weigert's basic-fuchsin-resorcin method.* Basic fuchsin 2 grams; resorcin or phenol 4 grams; water 200 cc. Boil 5 to 10 minutes. Add to the boiling mixture 25 cc. of a 30% aqueous solution of chlorid of iron ($FeCl_3$). Boil for 3 to 10 minutes. Then add drop-wise a saturated solution of the iron chlorid till the color is all precipitated. Test occasionally by letting a few drops run down the side of the beaker used for the heating. When the color is precipitated, it will appear as fine granules, and the liquid will be clear or slightly yellow.

When precipitated, allow the liquid to cool and the coloring matter to settle. Filter through filter paper. Scrape off the precipitate or cut off the lower end of the filter. Put in a pyrex beaker; add 200 cc. of 95% alcohol; heat over a water bath till the alcohol boils; stir the mixture occasionally with a glass rod; boil 5 minutes or more. Filter the hot solution into a pyrex beaker and allow it to cool. When it is cool, add with stirring 5 cc. of hydrochloric acid.

Stain paraffin or collodion sections in this one hour or less. Wash off the stain with 95% alcohol. This stain is good for any method of fixation. The elastic tissue will be dark gray or black. For multiple staining see § 661.

(3) *Sheridan's crystal-violet* (Stain Technology, Vol. 5, p. 31, 1935). This stain is prepared the same as Weigert's except that the crystal-violet is used instead of the basic fuchsin. Elastic fibers are stained green. Orange G is a good counterstain.

(4) *Verhoeff's iron-hæmatoxylin stain* (Jour. Amer. Med. Assoc., 1908, Vol. 50, Part 1, p. 876). 1 gram of hæmatoxylin crystals; 20 cc. of 95% alcohol. Heat in a test-tube till the stain is dissolved. When the hæmatoxylin is dissolved and the solution cool, add iron chlorid ($FeCl_3$) of a 10% aqueous solution 8 cc. Finally add 8 cc. of Lugol's solution. (Iodin 2 grams, iodide of potassium 4 grams, water 100 cc.)

Stain the sections from alcohol in this mixture till they are black (10–15 minutes or more), then differentiate with 2% aqueous iron chlorid solution. It takes half a minute or less to differentiate. If the differentiation is not sufficient the nuclei will be prominent; if it has been carried too far, the finest fibers will be too pale. They should be black. Wash with water, dehydrate, mount in balsam or clarite. If a counterstain is desired, picro-fuchsin (§ 610) is good.

Dr. Verhoeff says that the elastic stain with the Lugol's solution omitted is superior to alum hæmatoxylin for staining nuclei, and is very rapid. Sections are stained one minute or longer and then differentiated with two per cent. ferric aqueous ferric chlorid, then washed in water and counterstained with eosin or phloxine, and mounted in balsam or one of the synthetic resins.

This stain answers well for tissues fixed in any of the standard fixers. If the fixer contains mercury that may be removed in the usual manner by immersing the slide in iodized alcohol (§ 597).

(5) *Mallory and Wright's connective tissue stain* (§ 599) gives a bright pink color to the elastic tissue of the ligamentum nuchæ, and a blue color to its collagenous tissue.

In Dr. Mallory's new work, Pathological Technique, published in 1938, he says on page 153 that in preparing the connective tissue stain phosphomolybdic acid was originally used, but the acid fuchsin tended to fade. He now recommends the substitution of phosphotungstic acid, which, he says, gives more permanent preparations. In case the collagenous fibers are to be made as prominent as possible only the blue collagen stain is used, the acid fuchsin being omitted.

§ **583. Eosin.** — This is used mostly as a contrast stain with hematoxylin, which is almost a purely nuclear stain. It serves to stain the cell body, ground substance, etc., which would be too transparent and invisible with hematoxylin alone. If eosin is used alone, it gives a decided color to the tissue and thus aids in its study. Eosin is used in alcoholic and in aqueous solutions. A very satisfactory stain is made as follows: 50 cc. of water and 50 cc. of 95% alcohol are mixed and $\frac{1}{10}$ of a gram of dry eosin added. $\frac{1}{2}$% aqueous eosin is also good.

§ **584. Eosin in 95 per cent alcohol.** — For staining embryos and tissues so that the tissue in the ribbons of sections may be seen easily a saturated solution of alcoholic eosin is made. This is also used for staining with methylene blue.

§ **585. Eosin methylene blue.** — See Mallory & Wright, Pathological Technique, 8th edition, p. 102. This double stain is one of the most useful in microscopy. It is prepared and used thus:

Eosin soluble in alcohol only, or soluble in both alcohol and water.
Saturated solution of eosin in 95% alcohol.

Methylene blue, pure, such as is used in medicine	1 gram
Borax	1 gram
Water	100 cc.

For use dilute the methylene-blue-borax solution four or five times with water.

Stain the slide of sections in the eosin solution 3 minutes.

Wash the eosin off with plenty of water either by flooding with a pipette or by dipping the slide in a vessel of water.

Stain in the diluted methylene blue 10 minutes more or less.

Rinse off the excess dye in water.

Differentiate by pouring over the slide of sections, 95% alcohol till the sections begin to look pink. Rapidly dehydrate with absolute alcohol and clear with xylene; mount in balsam. It was pointed out by S. B. Wolbach (Jour. Amer. Med. Assoc., 1911, Vol. 56 (I), p.p. 345–346), that the addition of resin dark or light (colophonium) to the alcohol made the differentiation more precise and certain. He recommends the addition of 1% resin for Zenker-fixed tissue. For formalin-fixed tissue one may need to add from 5% to 10% of the resin. The use of denatured alcohol is also successful if one uses the resin.

§ **586. Ether, ether-alcohol.** — Sulphuric ether $(C_2H_5)_2O$ is meant when ether is mentioned in this book. Wherever ether-alcohol is mentioned it means a mixture of equal volumes of sulphuric ether and 95% or absolute alcohol, unless otherwise stated.

§ **587. Farrant's solution.** — Take 25 grams of clean, dry gum arabic, 25 cc. of a saturated aqueous solution of arsenious acid, 25 cc. of glycerin. The gum arabic is soaked for several days in the arsenic water, then the glycerin is added and carefully mixed with the dissolved or softened gum arabic.

This medium retains air bubbles with great tenacity. It is much easier to avoid them than to get rid of them in mounting.

§ **588. Flemming's Fluid.** — Water 19 cc.; 1% osmic acid 10 cc.; 10% chromic acid 3 cc.; glacial acetic acid 2 cc. This osmic fixer is good for very small pieces — 1 to 5 millimeter pieces, thickness not over 2 to 3 mm. Wash out with water 10 to 24 hours, then in 67% alcohol; later in 82% and 95%.

§ **589. Formaldehyde (HCHO or OCH_2).** — This is found in the market under the name of "formalin," etc., and consists of a 40% solution of formaldehyde gas in water.

For fixing tissues and embryos a 5% solution is good (formalin 1 cc., water 7 cc.). A common fixer is 10 cc. formalin, 90 cc. water. This is frequently called 10% formalin; it is, however, only 4% formaldehyde.

Tissues may stay in this indefinitely. Small pieces are fixed within an hour. Before hardening in alcohol and imbedding, wash out the formalin in running water half an hour, then harden a day or more in 67% and 82% alcohol.

For preserving nitric-acid-dissociated muscle a 2% formaldehyde solution is good. Formalin 1 cc., water 19 cc. (§ 558). See also § 538 for the formaldehyde dissociator.

§ **590. Glycerin. Glycerol** ($C_3H_5(OH)_3$). — (A) One should have pure glycerin for a mounting medium. It needs no preparation, unless it contains dust, when it should be filtered through filter paper or absorbent cotton.

To prepare objects for final mounting, glycerin 50 cc., water 50 cc., forms a good mixture. For many purposes the final mounting in glycerin is made in an acid medium, viz., glycerin 99 cc., glacial acetic or formic acid, 1 cc.

By extreme care in mounting and by occasionally adding a fresh coat to the sealing of the cover-glass, glycerin preparations last a long time. They are likely to be disappointing, however. In mounting in glycerin care should be taken to avoid air-bubbles, as they are difficult to get rid of. A specimen need not be discarded, however, unless the air-bubbles are large and numerous. See also congo glycerin (§§ 539–540).

§ **591. Glycerin jelly for microscopic specimens.** — Soak 25 grams of the best dry gelatin in cold water in a pyrex or agateware dish. Allow the water to remain until the gelatin is softened. It usually takes about half an hour. When softened, as may be readily determined by taking a little in the fingers, pour off the superfluous water and drain well to get rid of all the water that has not been imbibed by the gelatin. Warm the softened gelatin over a water bath and it will melt in the water it has absorbed. Add about 5 cc. of egg albumen (white of egg); stir it well and then heat the gelatin in the water bath for about half an hour. Do not heat above 75° or 80° C., for if the gelatin is heated too hot, it will be transformed into metagelatin and will not set when cold. Heat coagulates the albumen and it forms a kind of flocculent precipitate which seems to gather all fine particles of dust, etc., leaving the gelatin perfectly clear. After the gelatin is clarified, filter through a hot flannel filter and mix with an equal volume of glycerin and 5 grams of chloral hydrate and shake thoroughly. If it is allowed to remain in a warm place (i.e., in a place where the gelatin remains melted) the air-bubbles will rise and disappear.

In case the glycerin jelly remains fluid or semi-fluid at the ordinary temperature (18°–20° C.), either the gelatin has been transformed into meta-gelatin by too high a temperature or it contains too much water. The amount of water may be lessened by heating at a moderate temperature over a water bath in an open vessel. This is an excellent mounting medium. Air-bubbles should be avoided in mounting as they do not disappear.

§ 592. Glycerin jelly for anatomic preparations. — Specimens prepared by the Kaiserling method or other satisfactory way may be permanently preserved in glycerin jelly prepared as follows: Best clear gelatin, 200 grams; Kaiserling's No. 4 solution, 3000 cc. (Potassium acetate 100 grams; glycerin 200 cc.; water 1000 cc.) Put the gelatin in the potassium-acetate-glycerin-water mixture in an agate pail and heat over a gas or other stove. Stir. When the temperature is about 55° centigrade add the whites of three eggs well beaten, and stir them in vigorously. Make markedly acid by acetic acid. Continue the heating until the mixture just boils, and then filter through filter paper into fruit jars. It is best to put over the filter paper two thicknesses of gauze. A piece of thymol in the top of each jar will prevent the growth of fungi, or one can add 5% chloral hydrate. Specimens are mounted in this jelly directly from the No. 4 Kaiserling's, or alcoholic specimens can be soaked in water an hour or more and then kept in some of the melted jelly until well soaked; then they can be mounted permanently in the glycerin jelly. At the time of mounting the gelatin is liquefied over a water bath, and for every 20 cc. of the gelatin used, one drop of strong formalin is added. This is to prevent the liquefication of the gelatin after the specimen is mounted. Let the gelatin cool gradually after the specimen is in place, then add some melted gelatin to make the vessel over-full and slide a glass cover on it. This excludes all air. The cover may then be sealed with the clear gelatin or glue used for gluing wood, or the cement used in mending crockery. Finally, one can seal with rubber cement if desired. (See W. H. Watters, N.Y. Med. Record, Dec. 22, 1906.)

§ **593. Chloral hematoxylin.** — Potash alum 4 grams; distilled water 125 cc.; hematoxylin crystals $\frac{1}{10}$ gram. Boil 5 to 10 minutes in an agate or pyrex dish. After cooling, add 3 grams of choral hydrate and put into a bottle. This will stain more rapidly after a week or two if the bottle is left uncorked. It takes from 1 to 5 minutes to stain sections, — sometimes a long time. Use after any method of fixation.

It may be prepared for work at once by the addition of a small amount of hydrogen dioxid (H_2O_2).

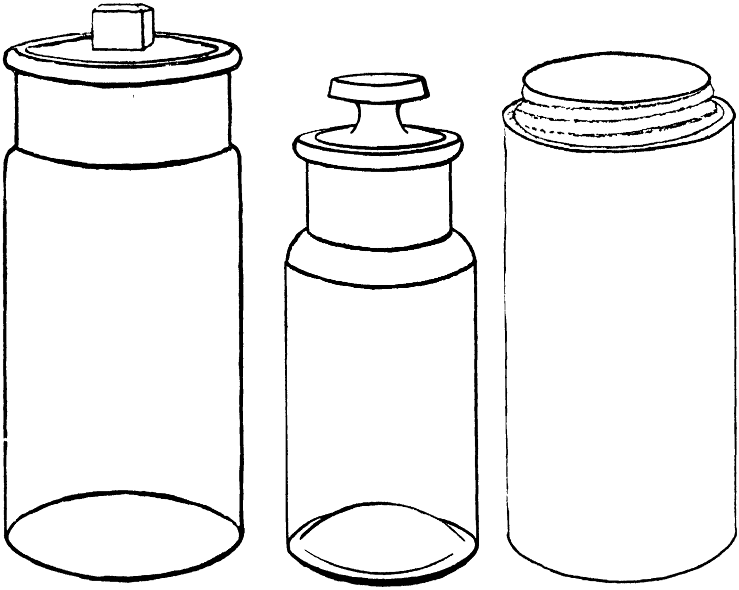
If the stain is too concentrated it may be diluted with freshly distilled water or with a mixture of water, alum and chloral. If the stain is not sufficiently concentrated, more hematoxylin may be added. (Proc. Amer. Micr. Soc., 1892, pp. 125–127.)

§ **594. Iron hematoxylin.** — For this stain there are three solutions: (*a*) the mordant composed of a 2% aqueous solution of ferric alum (iron-ammonium-persulphate); (*b*) a 0.5% solution of hematoxylin (10% alcoholic hematoxylin 5 cc., distilled water 95 cc.); (*c*) the differentiating fluid composed of the ferric alum diluted several times.

The stain can be used after any fixer, and the steps are as follows: (1) mordant with the ferric alum 1 to 24 hours; (2) rinse the specimen 10 to 30 minutes in water; (3) stain for 3 to 24 hours in the hematoxylin; (4) differentiate slowly, watching the effect under the microscope. For this, dip the slide into the ferric alum in the differentiator for a few seconds and then rinse with tap water. When satisfactory, wash in running water 15 to 60 minutes. The mordant and stain may be used several times.

§ **595. Hematein.** — This is used instead of hematoxylin, as it is believed to give more satisfactory results. Prepare as follows: Put a 5% solution of potash alum in distilled water and boil or leave in a steam sterilizer an hour or two. While warm, add 1 per cent of hematein dissolved in a small quantity of alcohol. After the fluid has cooled add 2 grams of chloral for each 100 cc. of solution. (Freeborn, Jour. Ap. Micr. 1900, p. 1056.)

§ **596. Iodin stain for glycogen.** — Iodin 1½ grams; iodid of potassium 3 grams; sodium chlorid 1½ grams; water 300 cc. For very soluble glycogen one can use 50% alcohol 300 cc. instead of water. The iodin stain is the most precise and differential for glycogen. Tissues or embryos for glycogen are fixed and hardened in 95% or absolute alcohol, and sectioned by the paraffin or by the collodion method. For permanent preparations the paraffin method is best (§ 640). In spreading the sections use this iodin stain instead of water. Glycogen in the sections stains a mahogany red, and the stain remains for ten or more years in the spread paraffin sections. Spread sections may be stained or restained by immersing the slide in iodin stain.



FIGS. 245–247. BOTTLES FOR FIXING AND PRESERVING TISSUES.
Fig. 245. Wide mouth specimen bottle with glass stopper.
Fig. 246. Salt mouth bottle with glass stopper.
Fig. 247. Glass jar with screw top.

Before mounting permanently, deparaffin with xylene, and mount in melted yellow vaseline. Press the cover down gently. Seal with shellac or balsam. (Trans. Amer. Micr. Soc., 1906, pp. 203–205.)

§ **597. Iodin in alcohol.** — Iodin 10 grams; 95% alcohol 90 cc. This is the strong stock solution.

For removing the pinlike or granular mercuric crystals from sections of objects fixed in any fixer containing mercury, e.g., Zenker's fluid, etc., take 95% alcohol 500 cc. and the 10% iodin solution 5 cc. In some cases, where the amount of mercury in the tissue is great, one may use 10 or even 15 cc. of the strong stock solution. Rinse the slide well in pure 95% alcohol to remove the iodin after all the crystals have dissolved (½ an hour or more).

For embryos and tissues fixed in a mercuric fixer one can add several drops of the stock solution to the alcohol containing the tissue and then by changing the alcohol occasionally the mercury will be mostly removed before sectioning. It is readily removed from the sections as just described.

§ **598. Lamp-black for ingestion by leucocytes.** — Lamp-black, 2 grams; sodium chlorid 1 gram; gum acacia (gum arabic) 1 gram; distilled water 100 cc. Mix all thoroughly in a mortar. The gum arabic is to aid in getting an emulsion of the lamp-black. Filter through one thickness of gauze and one of lens paper. If for a mammal, sterilize by boiling. If some of this mixture is injected into an animal, the leucocytes will ingest the carbon particles. Carmine may be used instead of lamp-black, but it is not so good because not so enduring as lamp-black.

§ **599. Mallory and Wright's connective tissue stain.** — Mallory and Wright, Pathological Technique, 8th edition, p. 118. Mallory, 1938 edition, p. 153. Two solutions are employed:

(1) Acid fuchsin (Rubine S) Certification No. 6	0.5 gram
Water	100.00 cc.
(2) Aniline blue, water soluble	0.5 gram
Orange G	2.0 grams
1% aqueous solution of phosphotungstic acid	100.00 cc.

Keep the solutions separate. Stain first with (1), 1 to 5 minutes, let the slide drain a moment and put directly in (2) without washing it.

Leave in (2) from two to four times as long as in (1). Every tissue seems to be a law unto itself, and one must find the best periods by experiment.

Rinse off the water and put directly into 95% alcohol or use a pipette and flood with 95%. Dehydrate, clear and mount in balsam. Collagenous tissue stains blue, elastic tissue red (§ 582, 5).

This is excellent for the ligamentum nuchæ, but not differential for elastic tissue, when there are many different tissues present.

§ **600. Mercuric chlorid** ($HgCl_2$). — Mercuric chlorid 7½ grams; sodium chlorid 1 gram; water 100 cc. The solution is facilitated by heating in an agate dish. Fix fresh tissue in this 2 to 24 hours. Then transfer to 67% alcohol a day or more and then to 82% alcohol. Tissues fixed in mercuric chlorid deteriorate; hence it is better to imbed them soon after they are fixed. Crystals of mercury are removed from the sections by the use of iodized alcohol (§ 597).

§ **601. Methylene blue, alkaline.** — Methylene blue 2 grams; 95% or absolute alcohol 50 cc.; distilled water 450 cc.; 1% aqueous caustic potash 5 cc. This stain works best after a fixer containing mercuric chlorid, like Zenker's fluid. (See § 584 for eosin in alcohol.)

§ **602. Mineral oil, pure, medicinal** (**petrolatum**). — The pure mineral oil used in medicine does not fluoresce and is of nearly the refractive index of glass (n_D 1.4815). It is good for mounting unstained sections, and for an immersion liquid. (See under the ultra-violet microscope (§§ 309, 536).

§ **603. Müller's fluid.** — Potassium dichromate 2½ grams; sodium sulphate 1 gram; water 100 cc. This is one of the oldest fixers. It must act a long time, two weeks to 10 or 12 weeks. This longer time is for nervous tissue to be stained for the myelin. Lately this fixer has been combined with mercury. (See Zenker's fluid, § 615.) Before putting the tissue into 67% alcohol it is washed out in running water for 24 hours.

Müller's fluid 10 cc.; normal salt solution 90 cc. forms an excellent dissociator for epithelia, etc. (§ 537).

§ **604. Neutral red.** — This is used especially for staining living animals. It is used in very weak solutions: $\frac{1}{10}$ gram red; 1000 cc. of water. Put a few cubic centimeters of this solution into the vessel containing the live animal, or animals. Infusoria stain quickly, 10 to 20 minutes or less. Vertebrates may require a few days. Try it on infusoria by adding a drop of the red to several drops of the infusion containing the infusoria. Be sure that there are many animals present. Watch them under the microscope and the color will be seen appearing in the granules of the infusoria. Then one may cover, and study with a high power (§ 543). For vital stains and the technique of their use see McClung, 74.81; Lee Microtomists Vade Mecum, 8th ed. Kingsbury, Histological Technique, and Kingsbury and Johannsen, Conn; Biological Stains, 2nd. edition.

§ **605. Nitric acid, HNO_3.** — This is employed for dissociation (nitric acid dissociator: water 80 cc., nitric acid 20 cc.); as a fixer, especially for chick embryos in the early stages (water 90 cc.; nitric acid, 10 cc.), and as a decalcifier (nitric acid 3 cc.; 67% alcohol 100 cc.).

§ **606. Normal liquids.** — A normal liquid or fluid is one which does not injure or change a fresh tissue put into it. The perfect normal fluids for the tissues of any animal are the fluids of the body (lymph and plasma) of the animal from which the tissue is taken. The lymph or serum of one species of animal may be far from normal for the tissues of another animal. (See also § 521.)

The commonly used artificial normal fluid is a solution of common salt (sodium chlorid) in water, the strength varying from $\frac{6}{10}$ to $\frac{9}{10}$ per cent. As indicated above, this normal salt or saline solution is employed in diluting dissociating liquids (§ 521).

§ **607. Paraffin wax.** — A histologic laboratory requires two grades of paraffin for ordinary work. These are hard paraffin, melting at about 54° centigrade, and a softer paraffin melting at about 43° centigrade. Usually a mixture of equal parts answers very well. It is economical for a laboratory to buy the paraffin wax in cases of about 100 kilograms.

All paraffin for imbedding and sectioning should be filtered through two thicknesses of filter paper. For this, use a metal funnel, heat the paraffin very hot in a water bath, and then heat the funnel occasionally with a Bunsen flame. The warmer the room, the easier it is to filter the paraffin.

Filter the paraffin into small porcelain pitchers. If the paraffin oven has a compartment large enough, it is well to keep one of the pitchers in the oven; then the paraffin remains melted and is ready for use at any time.

§ **608. Picric-alcohol.** — This is an excellent hardener and fixer for almost all tissues and organs. It is composed of 500 cc. of water and 500 cc. of 95% alcohol, to which 2 grams of picric acid have been added. (It is a $\frac{1}{5}$% solution of picric acid in 50% alcohol.) It acts quickly, in from one to three days. (Proc. Amer. Micr. Soc., Vol. XII (1890), pp. 120–122.) Not recommended for ultra-violet work.

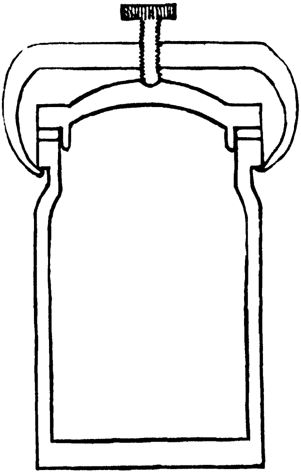


FIG. 248. SPECIMEN JAR WITH CLAMP.

§ **609. Petrolatum liquidum. See mineral oil (§ 602).**

§ **610. Picro-fuchsin.** — 10 cc. of a 1% aqueous solution of acid fuchsin; 75 cc. of a saturated aqueous solution of picric acid. Stain deeply with hematoxylin first; then use the picro-fuchsin. Wash off the picro-fuchsin with distilled water. Mount in non-neutralized balsam, or better in acid balsam (balsam 50 cc., glacial acetic acid 5 drops). If the white connective tissue is not red enough, increase the amount of acid fuchsin.

§ **611. Shellac cement.** — Shellac cement for sealing preparations and for making shallow cells is prepared by adding scale or bleached shellac to 95% alcohol. The bottle should be filled about half full of dry shellac; then enough 95% alcohol added to fill the bottle nearly full. The bottle is shaken occasionally and then allowed to stand until a clear stratum of liquid appears on the top. This clear, supernatant liquid is then filtered through filter paper or absorbent cotton, using a paper funnel (§ 566), into an open dish or a wide-mouth bottle. To every 100 cc. of filtered shellac 2 cc. of castor oil may be added to render it less brittle. The filtered shellac will be too thin, and must be allowed to evaporate till it is of the consistency of thin syrup. It is then put into a capped bottle. In case the cement gets too thick add a small amount of 95% alcohol or some thin shellac. The solution of shellac almost always remains muddy, and in most cases it takes a long time for the flocculent substance to settle. One can quickly obtain a clear solution as follows: when the shellac has had time to dissolve thoroughly, i.e., in a week or two in a warm place, or in less time if the bottle is frequently shaken, a part of the dissolved shellac is poured into a bottle and about one-fourth as much gasoline is added and the mixture well shaken. After twenty-four hours or so the flocculent, undissolved substance will separate from the shellac solution and rise with the gasoline to the top. The clear solution may then be siphoned off or drawn off from the bottom if one has an aspirating bottle. (R. Hitchcock, Amer. Monthly Micr. Jour., July, 1884, p. 131.)

If one desires to color the shellac, the addition of a strong alcoholic solution of some of the coal tar colors is good, but is likely to dissolve in the mounting medium when shellac is used for sealing. A small amount of lamp-black well rubbed up in very thin shellac and filtered is good to darken the shellac.

§ **612. Silvering.** — Intercellular substance stains brown or black with nitrate of silver. Use $\frac{1}{4}$ or $\frac{1}{2}$% aqueous solution on fresh tissue. Stain in the silver for 1 or 2 minutes; then expose to light in water till brown. Fix in 82% alcohol or 5% formaldehyde. One may stain afterward with hematoxylin for the nuclei; mount in glycerin, glycerin jelly, or in balsam.

§ **613. Sudan red, III for fat.** — Sudan III, aminoazo-benzene-β-naphthol $C_{22}H_{16}ON_4$) was introduced by Daddi into histology in 1896 (Arch. Ital. de Biologie, t. 26, p. 142), as a specific stain for fat. As it is soluble in all forms of fat and oils and in xylene, alcohol, etc., it is impossible to mount specimens in balsam after staining. As the fat of tissues is removed by the reagents used in the paraffin and collodion methods, only teased, free-hand, or frozen sectioned material, fresh or fixed in some non-fat dissolving fixer, can be used (Müller's fluid and 5% formaldehyde are excellent). The tissues cut free-hand or with the freezing microtome or teased can then be stained with a saturated alcoholic solution of the Sudan. It stains all fat a brilliant red. Preparations can be preserved in glycerin or glycerin jelly. This stain is largely used in pathology.

Daddi used the substance to feed animals and thus to stain the fat which was laid down in the body while the Sudan was fed.

The fat in the body already deposited remains unstained. This substance then serves to record the deposit of fat in a given period. In 1907 Dr. Oscar Riddle fed Sudan to laying hens, and the fat in the layers of yolk laid down during the feeding was stained red (Science, XXVII, 1908, p. 495). For staining the yolks of hen's eggs the hen may be fed doses of 20 to 25 milligrams of the sudan. Eggs so colored hatch as usual, and the chick, in utilizing the colored yolk, stains its body-fat pink (Susanna P. Gage).

Sudan IV or scarlet red is also used for a fat stain. See Gage, S. H. and Fish, P. A., Amer. Jour. Anat., Vol. 34, 1924.

§ **614. Table Black.** — During the last few years an excellent method of dyeing wood with anilin black has been devised. This black is lusterless, and it is indestructible. It can be removed only by scraping off the wood to a point deeper than the stain has penetrated.

It must be applied to unwaxed or unvarnished wood. If wax paint or varnish has been used on the tables, that must be first removed by the use of caustic potash or soda or by scraping or planing. Two solutions are needed:

Solution A

Copper sulphate	125 grams
Potassium chlorate or permanganate	125 grams
Water	1000 cc.

Boil these ingredients in an iron kettle until they are dissolved. Apply two coats of the hot solution. Let the first coat dry before applying the second.

Solution B

Anilin oil	120 cc.
Hydrochloric acid	180 cc.
Water	1000 cc.

Mix these in a glass vessel, putting in the water first. Apply two coats without heating, but allow the first coat to dry before adding the second.

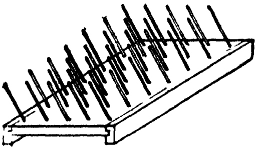


Fig. 249. Drying Rack with Inclined Pegs for Bottles.

When the second coat is dry, sandpaper the wood and wash it with water. When the wood is dry, sandpaper the surface again and then rub thoroughly with a mixture of equal parts turpentine and linseed oil. The wood may appear a dirty green at first, but it will soon become ebony black. An occasional rubbing with linseed oil and turpentine or with turpentine alone will clean the surface. This is sometimes called the Danish method, Denmark black or finish. See Jour. Ap. Micr., Vol. I, p. 145; Bot. Zeit., Vol. 54, p. 326; Bot. Gazette, Vol. 24, p. 66; Dr. P. A. Fish, Jour. Ap. Micr., Vol. VI, pp. 211–212.

§ **615. Zenker's fluid.** — Müller's fluid (§ 603) 100 cc.; mercuric chlorid 5 grams. Just before using, add 5 cc. of glacial acetic acid to each 100 cc. of the above. Fix fresh tissue 5 to 24 hours. Wash out with running water 24 hours. Then place in 67% alcohol 1 day or more and finally preserve in 82% alcohol. Tissue fixed in Zenker's has mercuric crystals. They may be removed from the tissue by long treatment with iodin, or by putting the slide bearing the sections in iodized alcohol for half an hour or more.

This is an excellent fixer, combining the good qualities of mercuric chlorid and of the chromium compounds. Tissues fixed with this show well the red blood corpuscles. This is called Helly's fluid if the acetic acid is replaced by 5% formalin.

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CHAPTER XII

FIXING AND THE PRESERVATION OF TISSUES, ORGANS, AND ENTIRE ORGANISMS. IMBEDDING, SECTIONING, STAINING, AND MOUNTING FOR THE MICROSCOPE

§§ 616–663; FIGURES 250–266

§ 616. Fixation and preservation of organs and tissues. — By fixing or fixation in histology is meant the preparation of fresh tissues, organs, embryos or small adult animals usually by means of some chemical mixture, called a "fixer," so that the organ, etc., as a whole and the elements or cells composing it shall retain as nearly as possible the morphologic characters present during life. The more perfect the fixer, the nearer will be the preservation of all structural details.

Unfortunately no single "fixer" preserves with equal excellence all the structural details, and therefore it is necessary to prepare the fresh tissue in several different ways and to make a composite of the structural appearances found, thereby approximating the actual structure present in the living body. Changes are so rapid after

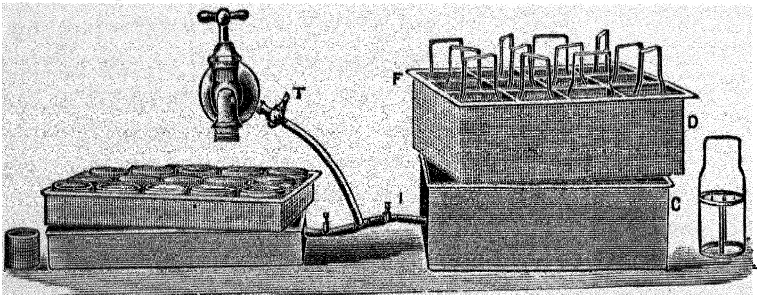


FIG. 250. WASHING BOXES FOR TISSUES FIXED IN A LIQUID CONTAINING MERCURIC CHLORID.

(From the Journal of Applied Microscopy).

T Small stop cock or pet cock in the usual water faucet so that a small stream may be drawn without interfering with the large faucet.

Only the larger trays are now used, the perforated inner tray being deep or shallow as needed.

death that the fixation should begin as soon as possible. For the most perfect fixation the living tissue must be put into the fixer.

With one of the larger animals, where the whole animal is to be used for microscopic study, it is a great advantage to bring the fixer in contact with all parts of the body quickly, and that is done by washing out the vascular system with normal salt solution and then filling the vascular system with the fixer. This method of *fixation by injection* is of great importance in the histology of animals which are large enough to inject.

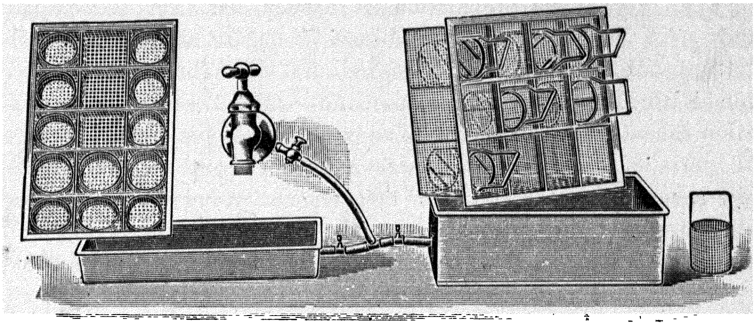


FIG. 251. METAL WASHING BOXES FOR TISSUES FIXED IN A LIQUID CONTAINING MERCURIC CHLORID.

(From the Journal of Applied Microscopy).

The deeper box is now used only and depending on the size of the pieces to be washed the shallow or the deep perforated trays and tissue baskets are used. The deep tray serves for washing slides with Weigert and other stains which must be in water a long time.

If the animal is too small for injection or one wishes only a small part of a larger animal, then the pieces for fixation should be small, say one to three cubic centimeters. Often, as for Flemming's fluid (§ 588) and for several others, it is better to use pieces 2 to 5 cubic millimeters in volume.

Large, solid organs must be cut into several pieces if the whole is needed. For hollow organs the cavity may be filled with the fixer and the organ placed in a vessel of the same.

The amount of fixer should be 10 to 50 times that of the piece of tissue.

Of the fixers given under "Preparation of Reagents," picric alcohol, formalin and Zenker's fluid are suitable for almost every tissue and organ. Formalin has the advantage of having strong penetration; hence it preserves whole animals fairly by immersing after filling the abdominal and thoracic cavities. Formaldehyde is excellent where a study of fat is in question, and it is much used as a fixer where frozen sections are desired (§ 625). Remember the necessity of removing mercury from sections of tissues fixed with a mercuric fixer (figs. 250–251).

§ 617. Mechanical preparation of tissues, etc., for microscopic study. — A limited number of objects in nature are small enough and transparent enough, and a limited number of the parts of higher animals are suitable for microscopic study without mechanical preparation except merely mounting them on a microscopic slide. Usually the parts of animals are so large and so opaque that the histologic elements or cells and their arrangement in organs can only be satisfactorily studied with a microscope after the tissue, organ, etc., have been teased apart with needles, or sectioned into thin layers.

MICROTOMES AND SECTION KNIVES

§ 618. The older histologists, those who laid the foundations and whose understanding of the finer structure of the body was in many ways superior to the knowledge possessed by workers at the present time, did their mechanical preparation with needles and with sharp knives held in the hand. They dealt also with fresh tissue more largely than we do at the present day, and learned also to distinguish tissues by their structure rather than by their artificial coloration.

What made them so successful was not, however, the lack of elaborate mechanical devices for sectioning and the complicated staining methods of the present day, but that they put intelligence and zeal into their work.

If the reader is interested in the mechanical means for sectioning he is referred to Dr. C. S. Minot's papers on the history of the microtome in the Journal of Applied Microscopy, Vol. VI, and to Gilbert Morgan Smith's article in the Transactions of the American Microscopical Society, Vol. XXXIV, 1915, on the Development of Botanical Microtechnique, pp. 71–129, 16 pages of bibliography; 18 figures, showing early microscopes and microtomes.

§ **619. Types of microtomes.** — There are two great types: (1) The early type in which the preparation to be sectioned is held mechanically and moved up by a screw, the section knife being held in the hand and moved across the object, usually with a drawing motion as in whittling.

(2) The mechanical type, in which both specimen and knife are mechanically held and guided, and the operator simply supplies power to the machine, or, when an electric motor is used, the operator starts and stops the machine and uses his hands in taking off the ribbon as it is cut. The ribbon is wound on a cylinder or cut into the proper lengths for the slide trays (figs. 237–238).

In the highest types of the second class — *automatic microtomes* — the operator needs only to put the knife and specimen in position and sections of any thickness and any number may be produced in a short time. A skilled and experienced person can get better results here as well as with free-hand sectioning or the hand microtome. Even automatic machines work better for skilled workmen.

In some forms the knife of these automatic microtomes is fixed in position and the object to be sectioned moves, while in other forms the object to be sectioned remains fixed and the knife moves. Furthermore, for sectioning paraffin, the knife meets the object like a plane (straight cut), while for collodion sectioning the knife is set obliquely and there results an oblique or drawing cut, as in whittling. For the latest models, see catalogues of the microscope manufacturers.

§ **620. Section knives.** — A section knife should have the following characters. (1) The steel should be good. (2) The blade should be slightly hollow ground on both sides. (3) The edge of the knife should be straight, not curved as in a shaving razor. (4) The back should be parallel with the edge. (5) The blade should be long, 12 to 15 centimeters, as it takes no more time or skill to sharpen a large than a small knife. (6) The blade should be heavy.

§ **621. Safety razor blades for sectioning.** — Recently the Bausch

& Lomb Optical Co., and the Spencer Lens Co., have put on the market holders for these blades that make them available as section knives in histology. The holders furnish the needed rigidity. Only about two millimeters of the cutting edge projects above the holder (fig. 252a). Extended personal use of these blades with the holders on the most varied material leads the author to recommend them strongly. They take away the time-consuming and tiresome labor of sharpening the large section knives. They are also recommended for much of the dissecting work. They may be held by the fingers, but preferably clamped to a handle by a small bolt.

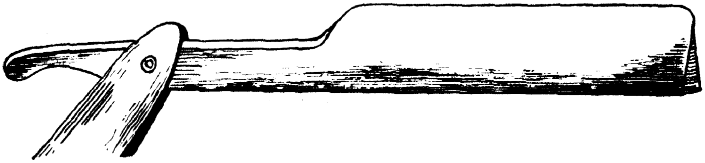


Fig. 252. Section Razor with Heavy Blade Having Straight Back and Edge.

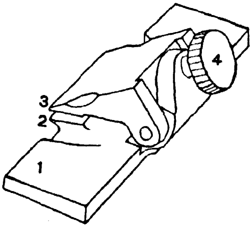


Fig. 252a. Safety Razor Blade Holder for the Microtome. (About one-third natural size).

1 Rigid metal the size of an ordinary microtome knife, to take the place of the knife in the microtome.

2–3 Jaws for holding the safety razor blade. It gives such firm support that practically only the cutting edge of the blade is free.

4 Screw head for tightening the jaws, 2–3.

As the cutting edge is not equally good in all the blades it is worth while to examine the edge under the microscope to see that it is smooth and free from nicks if one wishes to get the best thin sections, 5μ and less.

§ 622. Sharpening section knives; hones and strops. — Perhaps it should be taken for granted that any one would appreciate the impossibility of making good sections with a dull section knife, but experience teaches the contrary. Students are prone to believe that with one of the elaborate automatic microtomes, good sections may be made with any kind of an edge on the knife. It is forgotten that *the knife is the most important part;* all the other mechanism is simply its servant.

For sharpening, select a fine yellow Belgian hone, and a very fine Arkansas hone. As a rule hones from the factory are not sufficiently plane. They may be flattened by rubbing them on a piece of plate glass covered with moderately fine emery or carborundum wet with water. Round the corners and edges of the hones on the plate glass or on a grindstone. In using the Belgian hone for sharpening knives, wet the surface well with a moderately thick solution of soap. With the Arkansas stone use some thin oil — xylene or kerosene mixed with a little olive oil or machine oil.

Honing. Before honing a section knife, make sure that the edge is smooth; that is, that it is free from nicks. Test this by shaving off the surface of a block of paraffin. If nicks are present the cut surface will show scratches. It is advisable also to look at the edge of the knife with a magnifier and with a low power (48 (2x) mm.) objective. If nicks are present remove them by drawing the edge along a very fine Arkansas hone.

A saw edge may be all right for rough cutting and for shaving razors, but if one wishes to get perfect sections 1μ to 10μ in thickness a saw edge will not do. In removing the nicks one should, of course, bear on very lightly. The weight of the knife is usually enough.

In honing use both hands; draw the knife, edge foremost, along the hone with a broad, curved motion. In turning the knife for the return stroke, turn the edge up, not down. Continue the honing until the hairs on the arm, wrist, or hand can be cut easily or until a hair from the head can be cut within 5 mm. from the point where it is held. The sharper the knife becomes, the lighter must one bear on. One should also use the finest stone for finishing. If one bears on too hard toward the end of sharpening, the edge will be filled with nicks.

In honing and stropping large section knives, there has come into use during the last few years the so-called "honing backs." These elevate the razor slightly, so that the wedge is blunter and one does not have to grind away so much steel.

Strop. A good strop may be made from a piece of leather (horsehide) about 50 cm. long and 5 to 6 cm. wide, fastened to a board of about the same size.

The strop is prepared for use by rubbing into the smooth surface some carborundum powder, i.e., 60-minute carborundum, that which is so fine that it remains in suspension in water for 60 minutes, or one may use diamantine or jewelers' rouge.

Stropping. With the back foremost, draw the knife lengthwise of the strop with a broad sweep. For the return stroke turn the edge up as in honing. Continue the stropping until a hair can be cut 1 to 2 centimeters from where it is held. (See also the hones and strops and the methods of procedure recommended in the catalogues of microscopical manufacturers.)

§ **623. Free-hand sectioning.** — To do this one grasps the section knife in the right hand and the object in the left. Let the end to be cut project up between the thumb and index finger. One can let the knife rest on the thumb or index finger nail and, with a drawing cut, make the section across the end of the piece of tissue. By practice one learns to make excellent sections this way. If the whole section is not sufficiently thin, very often a part will be and one can get the information needed. The importance of acquiring skill in free-hand sectioning cannot be overestimated if one is to study living and fresh tissues, and without such study no one can gain a fundamental knowledge of structure. It is also of the highest value in the preparation of living and fresh objects for study with the polarizing and the ultra-violet microscopes.

§ **624. Sectioning with a hand or table microtome.** — The tissue is held by the microtome and moved up by means of a screw. The knife rests on the top of the microtome and is moved across the tissue by the hand. Microtomes of this kind are excellent. No one need wait for expensive automatic microtomes to do good sectioning. With a good table microtome, the knife being guided by the hand or hands of the operator, he can make straight cuts as for paraffin sectioning, or drawing cuts as for collodion work.

§ **625. Sectioning with a freezing microtome.** — In this method of sectioning the tissue is rendered firm by freezing and the sections are cut rapidly by a planing motion as with paraffin. Now the most usual freezing microtome is one in which the freezing is done with escaping liquid carbon dioxid. The knife should be very rigid. A carpenter's plane blade is often made use of. The tissue may be either fresh or fixed. If alcohol has been used, it must be soaked out of the tissue by placing it in water. Sometimes tissues are infiltrated a day or two in thick gum arabic mucilage before freezing. Drop a little thick mucilage on the top of the freezer, put the tissue in the mucilage, and turn on a small amount of carbon dioxid. It will soon freeze the mucilage and the tissue, as shown by the white appearance. When frozen, cut the tissue rapidly. It is well to have an assistant turn the feed screw up while the sections are cut. When 20 or 30 sections are cut, place them in water or normal salt solution. This is a rapid method of getting sections much used in pathology where quick diagnoses are demanded. In normal histology the freezing microtome is used mostly for organs or parts of greatly varying density. For example, if one wishes sections of the finger and finger nail, this apparatus offers about the only means of getting good sections. In that case the bone is decalcified before trying to make the sections (§ 580).

Frozen sections are also very useful for demonstrating the presence of fat by staining with Sudan III.

THE PARAFFIN METHOD OF SECTIONING

§ **626. Object of the paraffin.** — In the early periods in histology great difficulty was encountered in making good sections of organs and parts of organs, because the different tissues were unlike in density. At first tallow and beeswax, elder pith, liver, and various other substances were used to enclose or surround the object to be cut. This gave support on all sides, but did not render the object homogeneous. In the early sectioning, a great effort was made to keep all imbedding material from becoming entangled in the meshes of the tissue. This was guarded against by coating the object with mucilage, and hardening it in alcohol. This mucilage jacket kept the tissue free from infiltration by the imbedding mass and it was easily gotten rid of by soaking the sections in water.

A great advance was made when it was found that the imbedding mass could be made to fill all the spaces between the tissue elements and surround every part, the tissue assuming a nearly homogeneous consistency, and cutting almost like the clear imbedding mass. Cocoa butter was one of the first substances to be used for thus "infiltrating" the tissues. The imbedding mass must usually be removed before the staining and mounting processes; but in staining for glycogen by the iodin method, the stain is applied before the paraffin is removed (§ 596).

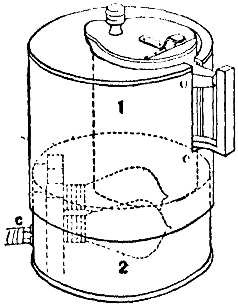


FIG. 253. KINGSBURY'S PARAFFIN MELTING OVEN. (From the Anatomical Record).

1 Upper part of the oven containing the covered pitcher for the paraffin.

2 Lower part containing the incandescent lamps and supply cable (*c*). The oven is well insulated by asbestos. Depending on the temperature of the room, one or both lamps can be used to keep the paraffin melted.

§ **627. Infiltration of the tissue with imbedding mass.** — The tissue to be cut in this way is first fixed by one of the fixers used for histology. Several good ones are given in sections 589, 608, 615, 616.

(A) The tissue is then thoroughly dehydrated by means of 95% and absolute alcohol. For most objects, especially embryos and other colorless objects, it is best, during the dehydration, first to use dilute alcoholic eosin (§ 583), as the most delicate part shows when one cuts the sections. Leave the piece of tissue to be cut overnight in alcoholic eosin, and a few hours in uncolored 95% alcohol, using 20 times as much alcohol as tissue. For the final dehydration it should be left in absolute alcohol four or five hours or overnight, depending on the size of the object.

(B) Remove the alcohol by a solvent of the imbedding mass; that is, by some substance which is miscible with both alcohol and the imbedding mass. Cedar-wood oil is most generally used, but pure xylene, chloroform, and carbol-xylene are also used, — the chloroform and carbol-xylene when osmic acid fat is to be retained in the tissue.

Leave the tissue in cedar oil or other clearer until the tissue sinks and the thin parts of the specimen become translucent. If the tissue does not sink after a time it means that the tissue was not dehydrated. Of course, this does not apply to lung or other spongy tissue containing much air. It is well to change the cedar oil or other clearer once. The used cedar oil may be left in an open bottle for the evaporation of alcohol and used over and over again.

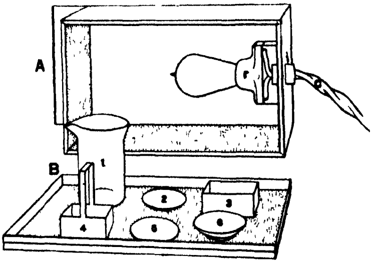


Fig. 254. Electric Infiltrating Oven with Projecting Spreading Plate.

(About one-eighth natural size. See also fig. 255–256.)

A Upper part of the oven with its brass spreading plate projecting 8 cm. to the left.

B Base or tray holding the oven, and the infiltrating and paraffin dishes. (1, 2, 3, 4, 5, 6.)

cr The electric cable and the porcelain receptacle for the lamp bulb.

The oven and tray are lined with asbestos, but there is none under the spreading plate. The dimensions are: Brass top, 38 × 18 cm., 3 mm. thick.

Oven, 30 cm. long, 18 cm. wide, 12.5 cm. high. Tray, 30 × 18 × 2 cm.

(C) Displace the cedar oil or other clearer by melted paraffin wax. When the tissue is saturated with the oil, transfer it to an infiltrating dish (fig. 254) containing melted paraffin. Place in a paraffin oven (fig. 254) and keep the paraffin melted for from two hours to three days, depending on the size and character of the piece to be imbedded. If the tissue is thoroughly dehydrated and well saturated with cedar oil, the melted paraffin permeates the whole piece. See § 641 for the propyl alcohol method.

§ 628. **Imbedding in paraffin wax.** — When the object is thoroughly infiltrated, imbed as follows: Make of strong writing paper a box considerably larger than the piece to be imbedded. Nearly fill the box with paraffin wax, place on a copper heater (fig. 260), and allow to remain until bubbles appear in it. Put the box on cold water until a thin stratum of paraffin solidifies on the bottom. Take the piece of tissue from the infiltrating dish (fig. 254) and arrange in the box for making sections in a definite direction. Add hot paraffin, if necessary, and then place the box on cold water. The more rapid the cooling, the more homogeneous will be the block containing the tissue to be cut. For the best imbedding it is well to drop 95% alcohol on the surface as soon as a film has formed in cooling. In warm climates where cold water is not easy to procure for cooling the blocks, one may float the paper box on 95% alcohol and with a pipette (fig. 264) drop strong alcohol on the sides of the box and on the top of the paraffin as soon as a surface film has formed.

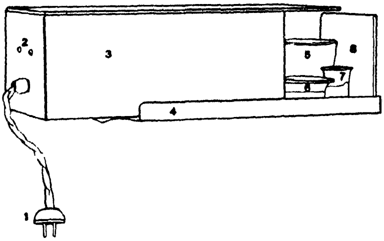


FIG. 255. INFILTRATING BOX AND SPREADING PLATE.

1 Connection for the electric circuit.

2 Screw heads. The screws hold the electric bulb socket in place.

3 Infiltrating box. It is 30 cm. long; 17.5 wide; 12.5 deep. The ends and sides are lined with thick asbestos. 3 mm. thick. The brass top, or spreading plate, is 17.5 cm. wide and 38 cm. long, i.e., it projects 8 cm. beyond the box (3).

5, 6, 7 Infiltrating and paraffin dishes

8 End of the box attached to the bottom (4). This infiltrating box adopts Dr. Kingsbury's plan of having the top and bottom sliding apart or together to regulate the temperature, and for ease in handling the infiltrating and paraffin dishes. G lamp bulb of 25 to 40-watt capacity supplies sufficient heat in a room at the ordinary temperature (20° centigrade).

It is very desirable to mark on the box the name of the imbedded object and to indicate which end or face is to be cut (§ 672).

§ **629. Fastening the block to a holder.** — Use one of the block holders or object discs furnished with the microtome, or a short stove bolt. Heat the larger end and press the paraffin block against the hot metal until it melts the paraffin. Hold the two together while cold water flows over them. When cold, the block is firmly cemented to the holder. Pains should be taken to have the axis of the block parallel with the long axis of the holder; and one should

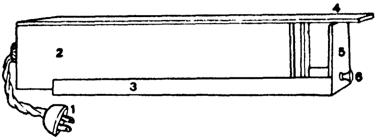


FIG. 256. DR. KINGSBURY'S HEATING BOX AND SPREADING PLATE.

1 Connection for the electric circuit.

2 Heating box, sides and ends lined with thick asbestos. Size of box: 30 cm. long; 16 cm. wide and 8 cm. deep.

3 The bottom of the box with the end (5) to close the box when the two parts are brought together.

4 The 8 cm. projection of the spreading plate. This plate is of brass, 3 mm. thick.

5–6 End of the heating box when the 2–3 are brought together, 6 is a knob to grasp in separating or putting together the sliding top and bottom (2–3).

The lower box is more convenient for spreading sections than the higher box (fig. 255), but not so large paraffin infiltrating vessels can be kept in it. The sliding feature of top and bottom enables one to control the temperature closely.

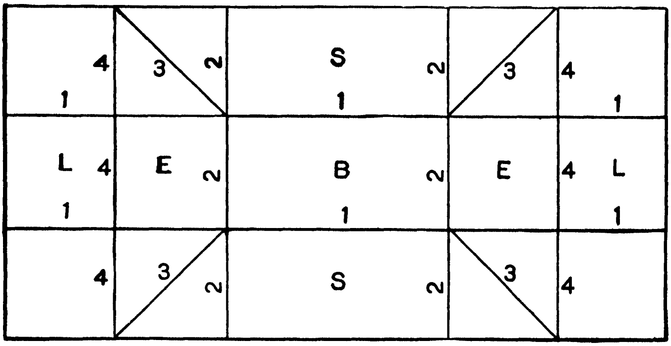


FIG. 257. DIAGRAM SHOWING HOW TO MAKE A PAPER BOX FOR IMBEDDING.

1 Lines for the first folds; these make three longitudinal strips.

2 Lines for the second folds; these make three transverse strips.

3 Lines showing where the corner folds are made.

4 The folds for the projecting end or label.

B Bottom, *S* Side, *E* Ends and *L* Label of the box. The bottom occupies ⅓ of the area.

not cut the block so short that the holder comes in contact with the tissue when the paraffin and holder are cemented together.

§ 630. **Fixing the imbedding block directly in the microtome.** With objects of considerable size, it is not necessary to fasten the imbedding block to a metal holder, and then to clamp that in the microtome, but the paraffin block itself can be put in the object clamp of the microtome.

§ 631. **Trimming the end of the block for sectioning.** — Sharpen the end to be cut in a pyramidal form, being sure to leave 2 millimeters or more of paraffin over the tissue at the end as well as on the sides. The block is trimmed in a pyramidal form, so that it will be rigid. Take particular pains that the opposite faces at the end of the block are parallel and all the corners right angles.

In some laboratories, Dr. McClung's for example, a cubical block of metal attached to a rod is placed in the knife holder of the microtome and the four sides of the imbedding mass trimmed with great exactness by the use of a straight-edged scalpel, or better by a small chisel, the cube of metal serving as a guide. As the metal cube can be slid along in the knife holder, and the imbedded tissue can be raised and lowered by turning the wheel of the microtome, imbedding masses of large and small sizes can be trimmed by the same metal guide. This guide for trimming is a great help in getting straight ribbons, and consequently good series.

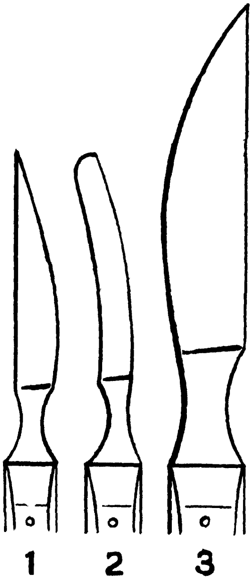


FIG. 258. SCALPEL BLADES. *1*, *2* with curved edges for cutting ribbons; *3*, with straight edge for trimming paraffin blocks.

§ 632. **Making paraffin sections.** — Put the paraffin block or the metal holder in the clamp of the microtome. Arrange the block so that one side of the pyramidal end is parallel with the edge of the knife; then tighten the clamp; and if an automatic microtome is used, make sure that the section knife is also tightly clamped by the proper set screws. It is well to have the knife lean slightly toward the paraffin blocks.

The knife edge meets the paraffin squarely, as in planing. The thickness of section is provided for in the automatic microtome by the indicator, which may be set for any desired thickness, or one can turn up the screw by hand in the table microtome. The paraffin and its contained tissue are cut in a thin shaving. If the tissue was stained *in toto* with eosin, as suggested in § 627 A, it is marked out with great clearness in the containing paraffin (§ 672).

As succeeding sections are cut, they push along the previous sections, and if the hardness of the paraffin is adapted to the temperature where the sectioning is done, the edges of the successive sections will be soldered as they strike. This produces a ribbon, as it is called, and if the paraffin block has been properly trimmed at the end the ribbon will be straight and even. If the ribbon is curved sideways, it indicates that one side of the block is thicker than the other and the sections are slightly wedge shaped.

If the paraffin is too hard for the room temperature and for a given thickness of section, the sections will curl; if it is too soft, the sections will crumple.

The thinner the sections, the harder should be the paraffin or the cooler the sectioning room; and the thicker the sections and the larger the object to be cut, the softer can be the paraffin and the higher the temperature. If, then, the sections do not ribbon, make thinner sections or work in a warmer place. If the sections crumple, make thicker sections or work in a cooler room. Of course, one can reimbed in a more suitable hardness of paraffin.

In the season when steam radiators are used, one can get almost any desired temperature by sectioning nearer or farther from the radiator.

In the winter it is a good plan to warm the microtome and section knife before sectioning. This can be done very easily by putting a cloth over the radiator and the microtome something like a tent.

§ 633. Electrification of the paraffin ribbons. — Some days there is such an accumulation of static electricity in cutting the ribbons that they jump toward anything brought near them. This is very annoying and likely to be so destructive to many of the sections that serial work cannot be done with safety.

Many devices have been tried to overcome this difficulty. One of the simplest and most successful is to put a pan of boiling water near the microtome or to boil some water near it. The water vapor given off in the surrounding air prevents fairly well the accumulation of static electricity, and the ribbons are thus free to remain where put. See also § 634 for Land's method.

§ **634. Land's method for sectioning hardened tissues.** — Some tissues like tendon, elastic tissue of the ligamentum nuchae etc. have a tendency to become so hard that it is practically impossible to get continuous ribbons. Dr. W. J. G. Land found that if the paraffin imbedded tissue in such a case had the paraffin pared away at one end until the tissue is exposed and then the paraffin block soaked in water for a day or more it is quite possible to cut continuous ribbons with ease. This was demonstrated on imbedded ligamentum nuchae that was so hard that the tissue was torn right out of the paraffin block when an attempt to cut it was made. The hard tissue was soaked in water for several days, and then sections, 3μ, 5μ, 7μ and 10μ were cut in perfect ribbons. This method, as also pointed out by Dr. Land, has the further advantage that the ribbons do not become electrified, and therefore that annoyance is also obviated. (See W. J. G. Land, Botanical Gazette, vol. 59, 1915, p. 401.)

See also remarks upon the method in Chamberlain's Plant Histology, 5th ed. p. 125 and 3d revised ed. p. 113. In this work, Dr. Chamberlain discusses and recommends the use of safety razor blades for section knives. (See § 621.)

§ **635. Storing paraffin ribbons.** — The most convenient method for caring for the ribbons as they are cut is to place them on a tray (figs. 237–238) lined with a sheet of white paper. It is important to write on the paper full data, giving the name of the tissue, the thickness of the sections, the date, etc. It is well also to number the ribbons and to indicate clearly the position of the first section or the beginning of the ribbon.

Ribbons of sections on a tray should be covered by another tray if one wishes to carry them to another room. The slightest gust of air sends them flying.

Ribbons on trays may be kept a long time, if they are stored in a cool place. The sections do not flatten out quite so well after standing a long time.

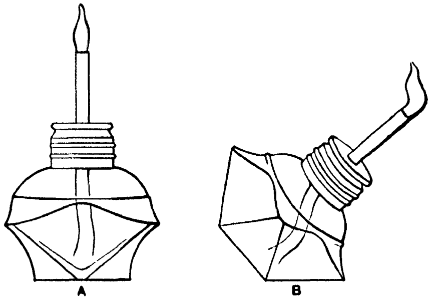


FIG. 259. ALCOHOL LAMP IN A VERTICAL AND AN INCLINED POSITION.

§ 636. Spreading the sections on water. — Paraffin sections are almost invariably slightly wrinkled or folded in cutting. To remove the wrinkles one takes advantage of the expansion of paraffin when it is warmed. The sections may be floated on warm water, when they will straighten out and become smooth, or the usual method is to stretch them on the slide upon which they are to be finally mounted.

By spreading sections on a wet slide a double operation is performed, viz.: the sections are made smooth and they are also fastened to the slide. Put a minute drop of albumen fixative on the middle of a slide and with the ball of one finger spread it over the slide, making a thin, even layer. It cannot be too thin. It is likely to stain if it is too thick. Do not use albumen if for the ultra-violet microscope (§§ 316, 318).

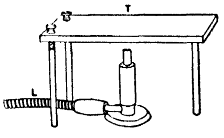


FIG. 260. LEVELING METAL TABLE FOR SPREADING SECTIONS AND FOR IMBEDDING IN PARAFFIN.

With a pipette (fig. 264) put several drops of water on the slide and then place a piece of ribbon on the water; or put the sections on the albumenized slide and add the water afterward. Heat the slide carefully over a spirit lamp or gas flame, being sure not to melt the paraffin. As the water warms, the paraffin expands and stretches the sections out smooth. A copper heating plate is good (fig. 260), but an electric spreader is best (figs. 254–256). The projecting top enables one to heat this oven with a gas or alcohol flame. If an electric bulb is used, one of 30 to 40 watts is sufficient. All desired temperatures are possible by placing the infiltrating dishes nearer or farther from the lamp; and in spreading one can pass from a point over the lamp where the paraffin may melt to the overhanging top which is only just warm. The dimensions of the oven giving optimum space and the desired range of temperature are about as follows: Length 30 cm.; width 18 cm.; height 12.5 cm. The brass spreading plate on top is 38 cm. long, 18 cm. wide and 3 mm. thick. The tray on the bottom is about 2 cm. deep. The tray and oven are lined with asbestos.

§ **637. Drying the sections.** — After the sections are spread, drain off most of the water, arrange the sections with a needle or scalpel, and place the slide in one of the trays (figs. 237–238). Allow it to remain overnight or preferably longer. The longer the drying in air, the more surely do the sections adhere to the glass slide; or use the drying oven (fig. 274).

If one is in haste to take the succeeding steps in the preparation, the slide may be dried by putting it into a drying oven at 38° to 40° C. for half an hour or more.

Some tissues are very difficult to get perfectly smooth, as just described. If fine wrinkles persist, one can sometimes overcome the difficulty by letting the slide cool and then covering with a piece of fine tissue paper slightly moistened; press down firmly with the ball of the finger on the sections. Then take hold of the edge of the paper and roll it off the sections.

As the water dries out, the spread sections come in very close contact with the glass and adhere quite firmly to it. The thinner the sections, the more tightly do they stick.

§ **638. Deparaffining in xylene.** — This is accomplished by using a solvent of paraffin. The best and safest one to use in a laboratory is xylene. Benzine, gasoline and even kerosene are used, but xylene is a powerful solvent of paraffin, does not injure the tissue, and is not very inflammable, on account of the large amount of carbon in its molecule (see § 573) and the consequent high boiling point, 136° C. (§ 244).

It requires only a few minutes to dissolve paraffin from the sections, but a day or more in the xylene does no harm.

When the paraffin is removed the staining and other operations necessary for a completed preparation may be undertaken.

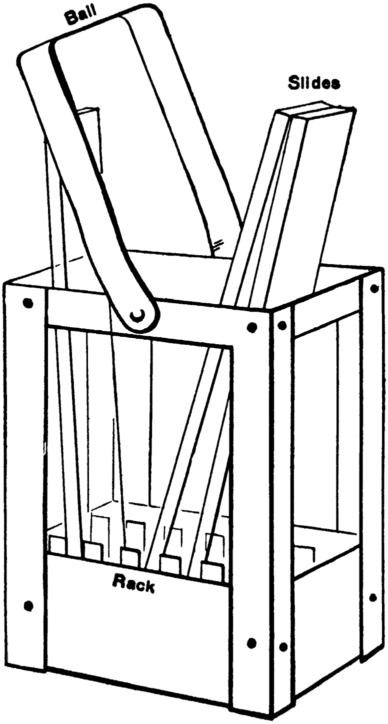


FIG. 261. SLIDE BASKET OR RACK FOR HANDLING SERIAL SECTIONS.

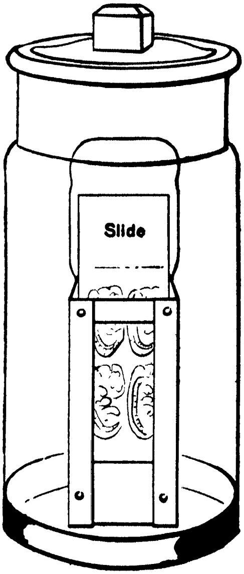


FIG. 262. GLASS STOPPERED SPECIMEN JAR WITH A SLIDE BASKET OR RACK WITH A SPECIMEN IN PLACE.

§ **639. Collodionizing the sections.** — Except for carmine stains and perhaps some others, collodion remains practically colorless. While the sections remain quite firmly attached to the slide after they have been spread and dried, thick sections are likely to come off in the many processes of staining, and if one has many sections on a slide, some of them may become loosened. To avoid this, the sections are covered with a delicate layer of collodion, which holds them down to the slide. The early method was to use a soft brush and paint a thin film over the dried sections before they were deparaffined. Now the sections are deparaffined, and then, after draining the xylene from the slide 10–15 seconds, it is put into a bottle containing $\frac{3}{4}$% collodion (§ 577). In a minute or more the collodion displaces the xylene, penetrates the sections and forms a delicate veil over their free surface. No harm is done by leaving the sections in the collodion a considerable time, but a minute or two is sufficient. The slide is removed, allowed to drain for half a minute, and then put into a jar of 67% alcohol (fig. 262). The alcohol fixes the collodion and removes the ether. As the 67% alcohol does not hurt the tissue, it may stay in the jar a day or more, if desired, but half an hour suffices.

The sections are now ready for the subsequent staining and other operations to make a finished slide. One has to remember that if mucicarmine (§ 570) is to be used in staining, the preparation must not be collodionized, as carmine stains collodion.

§ 640. Steps in order for the paraffin method. —

Name

Animal..............................
Date................................
Fixer...............................
Time of fixation....................
Washed in water.....................
67% alc..........82% alc...........
Decalc. § 580......67, 82% alc.....
In toto stain.....................
Washed in...........................
67% alc..........82% alc...........
95% alc. and eosin..................

No.

Absl. alc...........Cedar oil.......
Infilt. ....
Temp. bath......Imbed. in...........
Sections cut........μ's.........
Temp. room..........................
Stains..............................
....
Mtd. in.............................
Remarks.............................
....
....

§ 641. Paraffin sectioning by the propyl alcohol method (§ 563). Sheridan (Stain Technology V, 34) and Bradbury, Science, vol. 74, p. 225, have shown that there are certain advantages in the use of normal propyl alcohol ($CH_3CH_2CH_2OH$) and isopropyl alcohol ($CH_3CHOHCH_3$). They do not make the tissues so hard and brittle and the imbedded mass cuts easier.

Sheridan used normal propyl alcohol and the experience of the author has been with that quite extensively. The tissue is fixed in any desired manner as usual and carried up to 82% alcohol in the usual way. For the dehydration the normal propyl alcohol is used. When dehydrated it is passed directly to melted, 43° paraffin. It may remain in this with one or two changes for an hour or more, depending upon its size and character. The tissue is then transferred to melted paraffin of 50° to 56° melting point and left in it only a few minutes. If it is moved about the displacement of the soft paraffin by the harder paraffin will be facilitated. The tissue is then imbedded in the usual way and sectioned when convenient. It has proved highly satisfactory.

THE COLLODION OR PARLODION METHOD OF SECTIONING

In this method the tissue is thoroughly permeated with a solution of collodion, which is afterward hardened. Unlike the paraffin of the paraffin method, the collodion (§ 576a) is not subsequently removed from the tissue, but always stays in the sections. It is transparent and does no harm.

The fixing and dehydration with 95% alcohol is the same as for the paraffin method.

The paraffin method gives thinner sections than the collodion method and for series and large numbers of sections, is superior.

The collodion method requires no heat for infiltration, and it does not render the firmer forms of connective tissue so hard. It is especially adapted for making sections of large pieces of tissue or organs and when thick sections are desired. It is not easy to cut sections less than 10μ with collodion, while with paraffin it is possible to make good ribbons of small objects of delicate texture 2μ to 3μ in thickness. With a very sharp knife and small, delicate object, and one of the better forms of microtomes, one can cut short paraffin series in 1μ sections and get perfect ribbons.

In plant histology paraffin is used for cytologic work, and by many whenever possible. Collodion must be used for the hard tissues and is used by preference in some laboratories. (See references in the collateral reading at the end.)

Collodion sectioning is sometimes denominated the *wet method*, as the tissue and sections must always be wet with some liquid, while the paraffin method is called the *dry method*, as the tissue once infiltrated with paraffin keeps in the air indefinitely and in cutting the sections no liquid is used.

§ **642. Infiltration with ether alcohol.** — Transfer the piece of tissue to be cut from 95% alcohol to a mixture of equal parts of sulphuric ether and 95% or absolute alcohol, and leave in this for a few hours or a day or more, as is most convenient. This is to soak the tissue full of a solvent of the collodion.

§ **643. Infiltration with 1½% collodion.** — Pour off the ether alcohol from the tissue and add 1½% collodion. Leave in this overnight or longer if the piece of tissue is large.

§ **644. Infiltration with 3% collodion.** — Pour off the 1½% collodion and put in its place 3% collodion. Leave the tissue in this half a day or longer.

§ **645. Infiltration with 6% collodion.** — Pour off the 3% and add 6% collodion to the piece of tissue. For complete infiltration with this thick collodion, it requires one day at least. If the object is large, it is advantageous to infiltrate for a week or two.

§ **646. Infiltration in strong collodion.** — Many workers recommend as thick a solution as can be made for the final infiltration, and a long stay (2–3 weeks) in the infiltrating liquid.

Many also recommend a great many steps in the process, commencing with 1% and gradually passing up through increasing strengths till the thickest is reached.

§ **647. Imbedding on a cork or block.** — For imbedding small pieces use a piece of wood (deck plug), vitrified fiber, glass or good cork for a holder. Cover the end with 6% collodion and let it get well set in the air; then put the piece of tissue on the holder and drop 8% collodion upon it at intervals until it is well covered all around. If one takes considerable time for this, the collodion thickens greatly in the air. This is an advantage, for it gives a denser block for sectioning. After the collodion is pretty well set, place holder and tissue in a vessel with chloroform to harden. One can put the preparation into the chloroform, or, if the vessel is tight, it may be above the chloroform, the vapor then acting as the hardener.

§ **648. Imbedding in a paper box.** — If the object is of considerable size, it is best to use a paper box for imbedding, as with paraffin. If a very small amount of vaseline is rubbed on the inside of the box, it prevents the collodion from sticking to the paper (fig. 257, § 672).

Put first some of the thick collodion in the box and let it remain in the air until nearly solid, 2 to 3 minutes. Then arrange the specimen to be cut as for imbedding in paraffin, and gradually add thick collodion until the object is well covered. Let the box stand for a few minutes in air; then place it in a dish like a Stender dish and pour some chloroform on the bottom of the dish. Cover and the collodion will harden, partly by the chloroform vapor and partly by that which soaks through the paper. It is well to change the chloroform at least once. The used chloroform will contain some ether alcohol, but is good for killing animals.

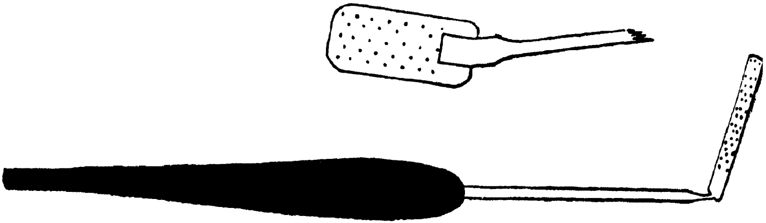


FIG. 263. PERFORATED SECTION LIFTER FOR HANDLING SINGLE COLLODION OR FROZEN SECTIONS.

After 24 or 48 hours the collodion should be firm all through. Then it is placed in 67% alcohol where it may be left a day or more. If it is to be left an indefinite time, the 67% alcohol should be changed for 82%.

§ **649. Sectioning by the collodion method.** — For this one can use a table microtome or one of the sliding microtomes. The sections are made with a knife set obliquely and hence with a drawing cut.

The holder with the small piece of tissue is clamped in the microtome and arranged as desired; then the sections are made with an oblique knife which is kept wet with 82% alcohol. The best way to keep the knife wet is to have a dropping bottle over the object, the drops falling about every two seconds. As the sections are cut, they are drawn up towards the back of the section knife with a soft brush. They can be kept in order in this way and do not interfere with succeeding sections.

Some operators in drawing the knife across the tissue use a slight sawing motion. However one proceeds, the knife is drawn rather slowly, not rapidly as with paraffin work.

If the imbedding was done in a paper box, remove the box and trim the collodion block suitably. Dry the end opposite the tissue, then wet it with 3% collodion. Use a piece of wood, a cork or other holder of suitable size. Put some 6% collodion on the holder and let it dry for a minute or so; then press the collodion block down on the holder. Leave in the air for a minute or two and then put into 67% alcohol to harden the cementing collodion. After 15 minutes, or longer if convenient, put the mounted specimen into the clamp of the microtome and cut as above.

Sometimes when the imbedded object is of sufficient size and the collodion block is firm, the block itself is put into the microtome clamp, no wood or cork holder being used.

§ 650. Transferring sections from the knife to the slide. — When one has cut the number of sections for one slide, they should be transferred to the slide as follows: Take a piece of white tissue paper about 3 × 6 centimeters in size and lay it on the knife over the sections. Press down slightly so the paper is in contact with all the sections. Take hold of the paper beyond the edge of the knife and gradually pull it down off the knife.

If there is the right amount of alcohol on the knife, the sections adhere to the paper and move with it. This transfers the sections from the knife to a piece of tissue paper. Place the tissue paper with the sections down on the middle of an albumenized slide. Cover with another piece of paper and press down gently. This presses the sections against the slide and absorbs a part of the alcohol. Take hold of one edge of the paper and lift it with a rolling motion from the slide. The sections should stay on the slide (§ 650a).

§ **650a.** — Various forms of paper have been used to handle the collodion sections. It should be moderately strong, fine-meshed, not likely to shed lint, and fairly absorbent. One of the first and most successful papers recommended is closet or toilet paper. Cigarette paper is also excellent. In my own work the heavy white tissue paper has been found almost perfect for the purpose. Ordinary lens paper or thin blotting paper for absorbing the alcohol or oil may be used with it.

§ **651. Fastening the sections to the slide.** — With a pipette, drop 95% alcohol on the slide of sections, then use a pipette full of absolute alcohol if it is at hand. Drain most of the alcohol away and add a few drops of ether alcohol. The collodion should melt and settle down closely on the slide. If the collodion does not melt the dehydration was not sufficient and more alcohol must be used. After the collodion has melted down upon the slide, let the slide remain a minute or two in the air, and then transfer it to a jar of 67% alcohol (fig. 262).

After half an hour or longer the preparation is ready to stain.

§ **652. The castor-xylene method of sectioning.** — The preparation of the tissue is the same as described in §§ 642–646, except that when the collodion is hardened in chloroform, the block is transferred to castor-xylene (§ 575). In a few days the collodion gets as transparent as glass and one can see the tissue within with great clearness. It can remain in the castor-xylene indefinitely.

In cutting one proceeds exactly as in § 649, except that the block is kept wet with castor-xylene, and not with alcohol. The sections are arranged on the knife and transferred to the slide in the same way as for alcohol sectioning (§§ 650–651).

For fastening the sections to the slide, as no water is present, one can add the ether alcohol at once. It is advantageous here to have a mixture of ether two parts and absolute alcohol one part for melting the collodion in these oil sections.

Allow the slide to remain in the air till the collodion begins to look dull; then the slide may be transferred to a jar of xylene to remove the oil. From the xylene it is transferred to 95% alcohol and then the slide is ready to be stained, etc., as described below (§ 654).

Steps in order for the collodion method. —

Name

Animal..............................
Date..............................
Fixer..............................
Time of fixation..................
Washed in water..................
67% alc.........82% alc..........
Decalc. § 580......... ....
67% alc.........82% alc..........
In toto stain........................
Washed in.........................
67% alc.........82% alc..........

No.

95% alc..........................
Ether-alc.........................
1½% col........3% col.............
6% col.........8% col...........
Imbedded.........................
Chloroform .. .67% alc............
Or castor-xylene.................
Sections cut ....μ's...........
Stains ....
Mounted in.......................
Remarks..........................

Double Imbedding in Collodion and Paraffin

§ **653. Need of double imbedding.** — Some objects like ova with considerable yolk and other objects in which the different parts are of unequal density or are very loosely bound together are advantageously imbedded first in collodion so that there will be a tough matrix to hold the parts in place, and then for ease and rapidity of sectioning, paraffin imbedding is added.

Steps in double imbedding:

1. Fix in any desired way.
2. Dehydrate with absolute alcohol half a day or more.
3. Put into ether alcohol half a day or more.
4. Put into ¾% collodion half a day or more.
5. Put into 2½% collodion 1 to 2 days.
6. Put into 5% collodion for one day or longer.
7. Imbed in the 5% collodion, using a paper box (fig. 257). Take the precaution to vaseline lightly the inside of the paper box (§§ 648, 672).
8. Float the imbedded tissue on chloroform in a glass dish.
9. When the collodion is hardened by the chloroform, remove the paper box and transfer to the castor-xylene (§ 575) clarifier to finish hardening and clarifying the collodion mass.
10. Put into melted paraffin for infiltration. Leave in the infil-

trating oven (fig. 254) a day or two. There is advantage, according to some, in transferring to pure xylene or to cedar-wood oil for half a day before putting into the imbedding paraffin. Section in ribbons as with paraffin (§ 632).

The sections are spread and stained exactly as for the paraffin method, except that carmine cannot be used without staining the collodion.

STAINING AND PERMANENT MOUNTING

§ 654. Generalities on stains. — From the standpoint of the object to be stained, dyes may be divided into two great groups:

(1) (*a*) Those which differentiate certain parts of the tissue and make them prominent. Such dyes are called then *differential* or *selective*. If the nucleus is the part selected, the dye is frequently called a *nuclear dye*.

(*b*) *General or counter stains*. These stain all parts of the tissue, and are usually contrasting in color; blue or purple and bright red are frequent combinations, e.g., hematoxylin and eosin. There is an appearance of differentiation even with a general stain, as the denser portions of the tissue seem more deeply stained; that is, there is more substance and more stain is taken up, hence the color is deeper.

(2) From the standpoint of the solvent used in preparing the stains they are called (*a*) *aqueous*, and (*b*) *alcoholic*.

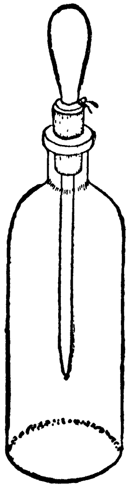


FIG. 264. REAGENT BOTTLE WITH PIPETTE.

If one uses an aqueous stain, the object must be well wet with water before the stain is applied, and afterward well washed with water before it is put again into alcohol. If an alcoholic stain is used, the object to be stained should be from alcohol of the same strength as that used in making the dye. The dye is also washed away from the tissue with the same strength of alcohol; it may then be put into the stronger alcohols for dehydration.

With reference to the now much used anilin dyes, Wright, Principles of Microscopy, p. 34, gives this excellent general statement: "Anilin dyes may be regarded as salts containing a coloring element or chromophore, united to a base or acid, according as the chromophore in question possesses, in the particular case, acid or basic properties. In the case where the chromophore functions as an acid, the dye is denoted an *acid dye* (e.g., eosin). In the case where the chromophore functions as a base, the dye is designated a *basic dye*." Eosin is used as an example where the chromophore functions as an acid, and methylene blue where the chromophore functions as a base.

The tissue elements and their parts are named from their affinity for acid or basic dyes. For example, in the blood, the red corpuscles and the granules of some of the leucocytes have an affinity for acid chromophores and hence stain strongly with eosin. They are accordingly said to be acidophil or oxyphil, sometimes also eosinophil. The nuclei of all the leucocytes, and of the red corpuscles when nucleated, and the granules of some of the leucocytes, have an affinity for basic dyes and hence stain with methylene blue, and are designated basophil.

§ **655. Staining with hematoxylin.** — Take a slide of sections prepared by the paraffin or the collodion method from the jar of alcohol and plunge it into a vessel of water to remove the alcohol. For staining put the slide of sections into a jar or shell vial of the hematoxylin solution, or lay the slide flat on the staining rack or some other support and add the stain to the sections (figs. 265–266). It usually takes from 2 to 10 minutes to stain sufficiently with hematoxylin.

A good plan when one is learning the process is to wash off the stain after one minute, either with a pipette or by putting the slide in a dish of water. Wipe off the bottom of the slide and put it under the microscope. Light well, use a low power, and one can see the nuclei stained a bluish or purple color, as hematoxylin is a nuclear dye. If the color is faint, continue the staining until the nuclei stand out boldly. Sometimes it takes a long time to stain well with hematoxylin. In such a case the jar of stain may be put into the paraffin oven and the heat will accelerate the staining.

One may also heat the individual slides as for spreading sections, but one must be careful not to let the stain dry on the sections. As the stain evaporates, add fresh stain with a pipette.

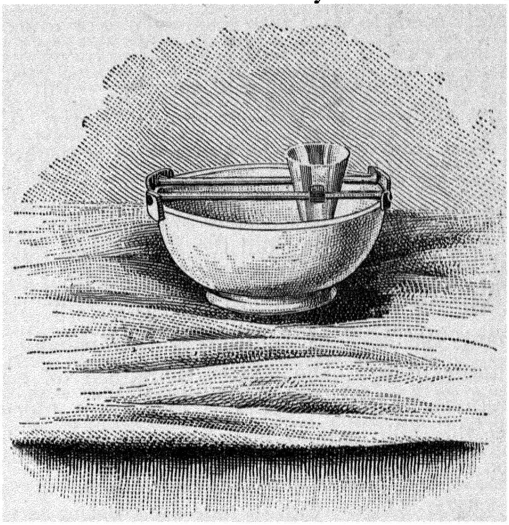


FIG. 265. BOWL WITH DRAINING RACK AND FUNNEL FOR STAINING SECTIONS.

When the sections are well stained with hematoxylin, wash off the hematoxylin with water. If the slide is allowed to stand some time in ordinary water the color is likely to be brighter. This is due to the action of the alkali (ammonia, etc.) usually present in natural waters. One could use distilled water, adding a few drops of a saturated solution of lithium carbonate.

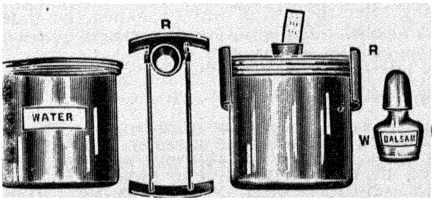


FIG. 266. SMALL AQUARIUM JARS FOR STAINING SERIAL SECTIONS.

R Rack for the top of the jar and containing a small draining funnel.

At the left is a spirit lamp used as a balsam bottle.

Dehydrate in 95% alcohol and absolute if necessary; clear and mount in balsam as described in § 535.

Hematoxylin is so nearly a pure nuclear stain for most tissues and organs that the cell bodies are not very evident with this alone; hence some counterstain is generally used also.

§ 656. Counterstaining with eosin. — One of the solutions of eosin (§ 583) is dropped upon the sections after the hematoxylin has been washed away with water. This stains almost instantly. One rarely needs to stain with eosin over 10 or 30 seconds. The excess stain is then washed away with a pipette or by dipping the slide into water.

§ 657. Dehydrating and clearing. — Put the slide directly into 95% alcohol after it is rinsed with water. Leave it in the alcohol a short time and transfer to fresh 95% alcohol or to absolute alcohol a few seconds, 10–20. One must not leave the sections too long in the alcohol or the eosin will dissolve out.

Remove the slide from the alcohol and put it into a jar of clearer (§ 573) or put it on the rack (figs. 265–266) and add enough clearer to cover the sections. Soon the clearer will displace the alcohol and make the sections translucent. It usually requires only half a minute or so. The clearer is drained off and balsam put on the sections, and then a clean cover-glass is added. One soon learns to use the right amount of balsam. It is better to use too much than too little (§ 535).

§ 657a. — In the past the plan for changing sections from 95% alcohol to water, for example, was to run them down gradually, using 75, 50 and 35% alcohol, successively. Each percentage might vary, but the principle of a gradual passing from strong alcohol to water was advocated. I have found that the safest method is to plunge the slide directly into water from the 95% alcohol. The diffusion currents are almost or quite avoided in this way. There is no time for the alcohol and water to mix; the alcohol is washed away almost instantly by the flood of water. So in dehydrating after the use of watery stains, the slide is plunged quickly into a jar of 95% alcohol. The diffusion currents are avoided in the same way, for the water is removed by the flood of the alcohol. This plan has been submitted to the severe test of laboratory work, and has proved itself perfectly satisfactory (1895–1931).

§ 658. Counterstaining with the eosin in the clearer. — With this method the eosin is dissolved in the carbol-xylene clearer (§ 573a), and the hematoxylin stained sections are dehydrated with 95% alcohol and absolute alcohol if necessary and then placed in the clearer. The sections are cleared and stained in eosin at the same time. It usually takes half a minute or more for the double process. When the sections are clear and sufficiently red, the slide is removed and the clearer drained off by holding in the forceps or in the draining funnel (figs. 265–266). Then the balsam is added, and covered as described above.

It is a good plan to rinse off the stained clearer by pure xylene before adding the balsam. This is not absolutely necessary, however.

§ **659. Hematoxylin and picro-fuchsin.** — Picro-fuchsin is so selective in its general staining that it is frequently used after hematoxylin. The hematoxylin staining should be intense and after the hematoxylin is washed away add the picro-fuchsin (§ 610). It takes only 10 to 30 seconds for it to act. Wash with distilled water, or natural water very faintly acidulated. The acid fuchsin is very sensitive to alkalies and fades easily.

Dehydrate in 95% and absolute alcohol, clear and mount in acid balsam. Acid balsam injures hematoxylin, but is necessary for the red in the picro-fuchsin.

Look out for mercuric chlorid crystals in the sections (§ 669).

§ **660. Hematoxylin and mucicarmine.** — Tissues and organs are best fixed in Zenker's, or mercuric chlorid. Small intestine is one of the most striking and instructive organs for this double stain. Make the sections by the paraffin method, but do not fasten them to the slide with collodion, for collodion stains with mucicarmine (§ 570).

Stain 1 to 24 hours in mucicarmine. Wash off the stain with water and then stain with hematoxylin. Do not stain too deeply. Wash with water, dehydrate, clear and mount in natural balsam. Nuclei will be bluish or purple and the cells containing mucus will be rose red. The goblet cells of the villi stand out like small red goblets, and if any mucus is streaming out of them, it will be red.

§ **661. Combined elastic mucicarmine and picro-fuchsin stain.** — For this, one should take some object that is known to contain elastic tissue, mucus, white fibrous tissue and muscle. (The non-cartilaginous part of the trachea is excellent.) The organ should have been fixed in mercuric chlorid or Zenker's fluid (§§ 600, 615) for this preparation. The sections should be made by the paraffin method and no collodion should be used for fastening the sections to the slide (§ 639), for collodion is stained by mucicarmine.

(1) Stain first in the elastic stain. Wash well with 95% alcohol and then with water.

(2) Stain in a shell vial or jar of mucicarmine (§ 570) from 1 to 24 hours. Wash well with water, but one must be careful in treating these sections, as they have no collodion mantle to protect them.

(3) Stain 15 to 30 seconds with picro-fuchsin of one-fourth strength (§ 610). Dehydrate with 95% and if necessary absolute alcohol. Clear in carbol-xylene and mount in acid balsam (§ 568). The elastic tissue will be black or blue black. Mucus will be carmine or rose red; white fibrous tissue will be magenta red; muscle, epithelium and blood will be yellow.

§ **662. Eosin methylene blue.** — One of the best objects for this stain is a hemolymph gland. Such a gland is easily and surely found by a beginner if he takes the heart and lungs of a veal. In the fat around the heart and behind the pleura will be found red bodies looking almost like blood clots. Remove carefully; fix in Zenker's fluid or mercuric chlorid (§§ 600, 615). Section by the paraffin method, make the sections 5μ and 10μ thick. Use collodion for insuring the fixation to the slide (§ 639). Stain with eosin methylene blue (§ 585).

Eosin-methylene blue staining is also excellent for demonstrating mucus.

Do not forget that mercury is likely to be present in sections of tissue fixed with any mercuric fixer. Remove it with iodized alcohol (§ 597). This should be done before the staining. One can tell whether the tissues contain mercury by looking at the unstained sections. The mercury looks black by transmitted light, white by reflected light. Seen by transmitted light, the substance is often in the form of delicate black pins.

§ **663. Iodin stain for glycogen.** — Use tissue fixed in 95% or absolute alcohol. Cut by the paraffin method. Mount the sections in serial order. Do not use water for spreading the sections, but one of the iodin stains for glycogen (§ 596). The glycogen will be stained at the same time that the sections are spread.

Let the sections dry thoroughly after spreading. Deparaffin with xylene and mount in yellow vaseline or use thin xylene balsam, but do not put a cover-glass over the balsam preparations.

The iodin stain remains in the spread sections for ten years or longer. One can restain any time by putting the slide with the spread, but not with the deparaffined sections, in a shell vial of the iodin stain. It is possible also to stain the nuclei with hematoxylin in the same way. If this is done, the hematoxylin should be used first and washed off with water and the iodin stain be used last, but not washed off with water.

For collateral reading see the references given in the preceding chapter (Ch. XI).

CHAPTER XIII

SERIAL SECTIONS OF ORGANS, SMALL ANIMALS AND EMBRYOS; PREPARATION OF MODELS; EXAMPLES AND EXPERIMENTS

§§ 664–702; FIGURES 267–277

ADVANTAGES OF HISTOLOGICAL SERIAL SECTIONS

§ **664. General on series.** — It is coming to be appreciated more and more that in histology as well as in embryology one can only get a complete knowledge of structure by having the entire organ cut in microscopic sections and each section mounted in order. Furthermore, it is necessary to have the organ cut in three different planes. In this way one can see every aspect of the structural elements and their arrangement in the organs.

In single sections one gets only a partial view. For example, how many students have any other idea of a ciliated cell than that it is a cell with triangular outline with a brush of cilia at the broad end. Probably many would be puzzled if they had a top view of the ciliated end; and the attached end would be even more puzzling.

It may not be possible for every worker to make serial sections of all the organs in all the three planes, but every one who is working seriously in histology can make all his preparations serial; that is, the sections which are mounted can be in serial order; then a puzzling appearance in one section may be perfectly intelligible in one a little farther along.

To get the greatest benefit from serial, as indeed also from single sections, the sections should be made in a definite manner; that is, they should be exactly across the long axis of an organ or parallel with the long axis (*Transections* and *Longisections*).

Or with such an organ as the liver, the skin, etc., the sections may be parallel with the surface (*Surface Sections*) or at right angles to the surface (*Vertical Sections*).

§ **665. Order of serial sections.** — Some plan must be adopted in arranging the series or only confusion will result. An excellent plan is to arrange the short pieces of ribbons for a given slide as the words on a page are arranged. That is, section No. 1 is at the upper left-hand corner. The next row of sections begins where the first row left off, etc. (fig. 267).

As the paraffin stretches considerably one must cut the ribbons into pieces considerably shorter than the cover-glass to be used.

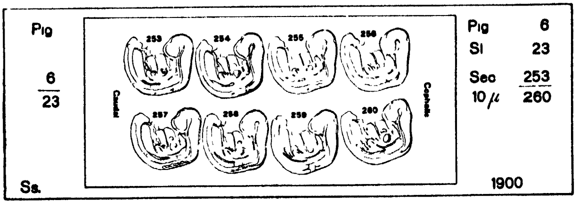


FIG. 267. A SLIDE OF SERIAL SECTIONS SHOWING THE ARRANGEMENT AND ORDER OF THE SECTIONS; ALSO THE LABELING OF THE SLIDE.

Both the paraffin and collodion methods are adapted to the preparation of series. The paraffin ribbons are easier to manage and easier to make than the serial sections in collodion.

By arranging the collodion sections as they are cut on the knife in collodion sectioning (§ 649), one can put them on the slide in perfect series by the tissue paper method (§ 650).

If the sections are large, as in cutting serial sections of the central nervous system, the series can be kept in order in a small dish by putting a piece of tissue paper over each section and piling them up. If the vessel is small enough, the papers and sections will not shift and get out of order. Or one might put a single section in a Syracuse watch glass or a Petri dish. Then in mounting, the sections can be taken in order.

§ **666. Numbering the serial slides.** — For temporary numbering a fine pen with Higgins' or Weber's waterproof carbon ink serves well. If the end of the slide is varnished, one can write on it as well as on paper. When the ink is dry it should be coated with thin xylene balsam or with any good varnish like valspar 1 part, xylene 9 parts. It is also important to write the number of the slide with a writing diamond. The double marking is desirable because with wet slides the diamond number is hard to see, while the ink marks are clearly visible. One is not so likely to wipe off the sections if the ink mark is present.

Fixing and Staining for Series

§ **667. Fixing.** — The two most used fixers for embryos are Zenker's fluid and formaldehyde (§§ 589, 615). For those unskilled in microscopic technic, or for one who is exceedingly busy, the best results are obtained by putting the embryos in formaldehyde (10 parts of formalin, the formalin of the pharmacy, and 90 parts water answers well). If there is plenty of this the embryos are likely to be well preserved even though they are left in the membranes, and that is far the best way for small embryos.

§ **668. Fastening the sections to the slide.** — For all serial work it is especially desirable to fasten the sections to the slide with collodion (§ 639). This should always be done unless some stain like carmine is to be used on the slide after the sections are fastened. With thin sections, if one is careful enough, an entire series can be carried through without losing a section, but with thick sections (15μ and thicker) some are almost sure to separate from the slide if not fastened by collodion.

§ **669. Removal of mercuric chlorid from sections.** — It should be remembered that if a fixer containing mercuric chlorid is used, the sections are almost sure to contain mercury. By transmitted light the mercury appears dark. Often the appearance is as if a multitude of delicate black pins were in the section. Sometimes the mercury is in rounded masses. This should be removed by putting the slides of sections into alcoholic iodin (§ 597). After half an hour or an hour, wash off the iodized alcohol with pure 95% alcohol and the sections are ready for staining.

If the embryo was stained *in toto* and contains mercury, the sections should be passed from the deparaffining xylene to the iodized alcohol (§ 597). After half an hour or more the slides are passed through pure 95% alcohol, and back to the xylene or to carbol-xylene. Then they can be mounted in balsam.

§ **670. Staining for series.** — There is a great advantage in point of time and safety in staining the entire embryo in some good stain like borax carmine (§ 569). Carmine is a very permanent stain. For bringing out special structural details the sections are stained on the slide as described in §§ 655–656. The slide baskets are almost a necessity for serial work (figs. 261–262), as the slides are handled individually only twice, (1) when they are spread and dried and put into the baskets, and (2) after all the processes are complete and the sections are to be mounted in balsam.

The sections are mounted in balsam directly from the deparaffining xylene. No alcohol is used unless it is necessary to remove crystals of mercuric chlorid (§§ 597, 669).

COMPLETE SERIES OF EMBRYOS AND SMALL ANIMALS IN THE THREE CARDINAL PLANES, — TRANSECTIONS; SAGITTAL SECTIONS; FRONTAL SECTIONS

§ **671. Serial sections of entire animals.** — With improvement in means for making thin sections of objects, the long-desired ability to see the entire organism in complete series is now easily realized. What was formerly determined with so much difficulty in dissecting embryos can now be attained with ease in a complete series. It is almost too easy, and with a lively imagination structural arrangements are described and depicted which never actually existed in the animals or embryos themselves. It is so difficult for most people to add the third dimension accurately when working with flat specimens that it is now appreciated that the older workers had a great advantage in dissecting the entire animal or embryo because they were there dealing with an obviously three-dimensional object and true relations in space were seen. There is now a wholesome tendency toward the retention of the advantages of dissection of entire forms with the advantages of serial sections. Hence embryos are now dissected entire almost as much as in the old days, and enlarged models of the series are made so that the object can be seen in three dimensions, the models also serving to make it easy to follow out the relations of parts with the naked eye. But one should not forget that a model, like a drawing, is after all only the interpretation of the artist and the thing itself must be referred to whenever there is to be real advancement in knowledge. Furthermore, as it is not possible to both dissect and serial section the same objects, and sometimes very few are available, anatomists have decided on the three planes which give the greatest information, — transections or cross sections, sagittal sections and frontal sections. With sections in these three spatial planes it is possible to gain some just conception of the actual relation of parts and structures in the object.

§ **672. Orientation of imbedded objects.** — In order that sections may be made in any desired plane the object must be so arranged or *oriented* in the imbedding mass that one can attach the imbedding block to the microtome holder, and then arrange for sectioning in a definite manner. With translucent or transparent collodion where the position of the object can be seen after it is imbedded, this is not particularly difficult, but with paraffin, which is nearly opaque, one cannot see distinctly enough the position of the object to give the exact arrangement necessary to make precise sectioning possible. The embryo or animal or other object must, therefore, be arranged in the imbedding box in a very definite manner.

To overcome the difficulties Dr. Kingsbury, ten to fifteen years ago, devised the method of making a diagram of the object to show its exact shape and position. (Anat. Record, Vol. XI, 1916, p. 294). The method is as follows: A natural-size diagram of the object is made on the inside of the bottom of the imbedding box before any paraffin is put into it. This is most easily done before the box is folded, or the folded box can be unfolded and made flat again. For making the diagram a soft lead pencil can be used or one of the ordinary colored crayons or a colored glass pencil. In any case enough of the lead pencil or the crayon mark adheres to the paraffin to make a clear diagram on it of the object.

In imbedding, the object should be arranged exactly over the diagram. The solidified layer of paraffin formed before the object is placed in the box (§ 628) is no hindrance, as the diagram shows through it clearly.

For embryos and small animals, of which serial sections are to be made, there should always be a photograph natural size.

The diagram for orientation is easily made from such a photograph by the use of the drawing shelf (fig. 277, A.D.S., §§ 415, 417). As the embryo or animal is always imbedded with the right side down, left side up, one must be sure to have the diagram in the same position. This is easily accomplished, as one can draw equally well with the photographic print whichever side is up. That is, if the embryo was photographed left side down, the print should be face down on the drawing shelf to bring the diagram in the imbedding box with the left side up. On the other hand, if the photograph was made with the embryo right side down, then the print should be face up when making the diagram on the bottom of the imbedding box.

With the definite outline of the embryo or animal on the bottom of the imbedding mass one has a good guide for arranging the object for sectioning any desired plane.

§ **673. Thickness of serial sections.** — The thickness of the sections of a series should be known in all cases; and for modeling it is absolutely necessary (§§ 680, 684). The thickness usually depends somewhat upon the size of the object to be made into series. If the object is small, the sections can be thin without having an unmanageable number of slides. With larger objects the sections are naturally made thicker to keep the length of the series within bounds.

One of the following thicknesses will be found to meet nearly all requirements and make modeling easier than as if some odd number of microns were used: 5μ, 10μ, 15μ, 20μ, 25μ, 30μ, 40μ, 50μ, 75μ, 100μ. Of course every investigator decides for himself the thickness of section which will serve his purposes best.

§ **674. Arrangement of sections on the slide.** — (1) A satisfactory and widely adopted method is to arrange the sections like the printed words in a book. This brings the first section at the upper left-hand corner of the series, and the last section at the lower right-hand corner (fig. 269).

(2) It is a great advantage to have the sections so arranged on

the slide that under the compound microscope the aspects will be as in the observer's body; then it will be easy to locate objects at the right or left, dorsal or ventral.

(3) Remember that in the ribbons the surfaces are somewhat unlike in appearance. The lower surface, that is, the surface facing the section knife, is shiny, while the opposite surface is dull. This knowledge is important, for sometimes sections get turned over accidentally. It is unfortunate to have part of the sections of a series wrong side up.

(4) The aspect cut first will face upward on the slide; that is, if the head is cut first the cephalic aspect will face up; if the left side is cut first the sinistral aspect will face up, and if the dorsal side, the dorsal face will be up.

(5) The aspect of the embryo which first meets the edge of the knife will be at the beginning of the series. If arranged and cut as here directed, transections would have the right side of each section toward the left on the slide (fig. 269). Under the compound microscope it would appear on the right.

For sagittal sections where the caudal end meets the knife, the caudal end of the section would be toward the left on the slide (fig. 272).

For frontal sections (fig. 270) where the right side meets the knife edge first, the right side of each section will be toward the left end of the slide.

§ 675. Mounting. — Cut the ribbons into segments of equal length, using preferably a curved knife (fig. 258). Transfer to albumenized slides with fine forceps (fig. 221). Make parallel with the long axis of the slide, and put the first section at the upper left-hand corner (fig. 267).

In a word, decide on some good plan for mounting series and follow the plan consistently.

§ 676. Size of slides and cover-glasses for series. — (1) If the object is small, the standard slide 25 × 75 mm. (fig. 217) is good and the cover-glass can be either 22 or 23 mm. wide and 50 or 60 mm. long. The smaller sizes are to be preferred when convenient, for more space is left to the label, and the cover-glass is not too near the edge as with wide covers.

(2) If the embryo or animal is of moderate size, that is, not over 30 to 35 mm. long, one can use advantageously the intermediate size of slides (fig. 216), that is, those 38 × 75 mm. A suitable cover-glass is 35 × 50 or 35 × 60 mm.

(3) For objects of considerable size, i.e., over 35 mm. in length, if sagittal or frontal sections are to be made, and if they are to be mounted crosswise, the slide must be of sufficient width. Ordinarily the large standard, 50 × 75 mm., will answer (fig. 215). For the large slides the covers can be 48 × 60 or 48 × 65 mm. For special large sizes of object, special slides can be made of lantern slide covers or old negative glass, etc., and for cover-glasses one can go back to the earlier workers and use mica.

Do not use too thick cover-glasses, or high powers cannot be employed in studying the sections (§§ 101–106).

TRANSECTIONS OR CROSS SECTIONS

§ **677. Transections** are those made by dividing the body into sections made across the long axis of the body. This divides the embryo or animal into equal or unequal cephalic and caudal segments. With microscopic sections, of course, the segments of the entire body are very unequal, although each section may be of equal thickness.

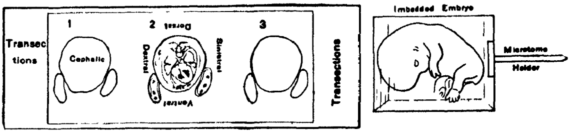


FIG. 268. SERIAL TRANSACTIONS.

At the right is the embryo in the imbedding mass and attached to the microtome holder.

At the left is a glass slide showing how the sections are to be mounted.

Imbedded embryo It is in the proper position for transections.

In section *1*, the word cephalic shows that the section is cephalic face up; the caudal face rests on the slide. In the middle section the words indicate the edges of the section. Under the microscope the words will be erect. Invert the book and the appearance will be the same as under the microscope.

(1) Imbed the embryo or animal with the right side down, taking

the precaution to have a layer of partly solidified paraffin at the bottom of the box (§ 628); and arrange the object exactly over the orientation diagram in the bottom of the imbedding box (§ 672).

(2) Mount the block of paraffin containing the embryo so that the caudal end is next the microtome holder. The head is then cut first, and the caudal surface of the sections will rest upon the slide, bringing the cephalic face up (fig. 268).

(3) Place in the microtome so that the right side of the embryo or animal meets the edge of the knife.

(4) Mount the sections like the words in a printed line. This will bring the first or most cephalic section at the upper left-hand corner. The cephalic face will be up, and the dorsal aspect next the upper edge of the slide.

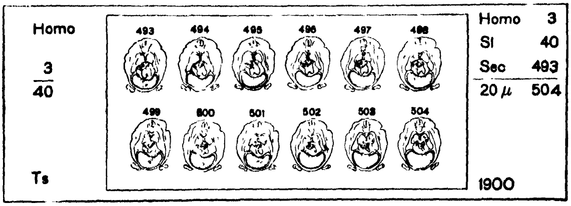


FIG. 269. A SLIDE OF SERIAL TRANSECTIONS SHOWING THE ARRANGEMENT AND THE LABELING OF THE SLIDE.

Under the compound microscope the rights and lefts will appear as in the observer's own body, as will also the dorsal and ventral parts.

FRONTAL SECTIONS

§ **678. Frontal sections.** — These are sections made by dividing the body into equal or unequal dorsal and ventral parts.

(1) Imbed the animal or embryo with the right side down in the imbedding mass (§ 628); and arrange the object exactly over the orientation diagram in the bottom of the imbedding box (§ 672).

(2) Mount the block of paraffin containing the embryo so that the ventral aspect of the embryo or animal is next the disc of the microtome holder (fig. 270). The dorsal part is then cut first, and

the ventral surface of the sections will rest upon the slide, bringing the dorsal face up.

(3) Place in the microtome so that the right side of the object meets the edge of the knife first.

(4) Mount the sections like the words in a printed book. This will bring the first or dorsal section in the upper left-hand corner of the series. The dorsal face will be up, the right side to the left, and the cephalic end toward the lower edge of the slide (figs. 270–271). Under the compound microscope the cephalic end will be away from the observer or in front, and the rights and lefts will be as in his own body.

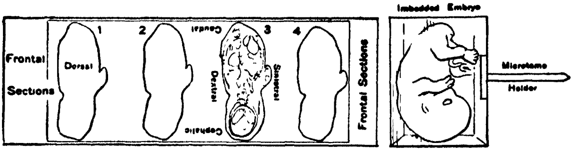


Fig. 270. Frontal Serial Sections Showing the Arrangement of the Embryo in the Imbedding Mass, the Connection with the Microtome Holder and the Position of the Sections on the Glass Slide.

Microtome Holder The metal disc and stem for holding the imbedded embryo in the microtome while sectioning.

Imbedded Embryo The embryo in the proper position for frontal sections.

Frontal Sections A slide showing the proper arrangement of frontal sections.

1, 2, 3, 4 Serial order in which the sections are arranged like the words in a printed book.

In section *1* the word dorsal indicates that the section has its dorsal face upward away from the slide while the ventral face is down in contact with the slide.

In section *3*, the words cephalic, caudal, dextral, sinistral are wrong side up so that they will appear erect under the compound microscope.

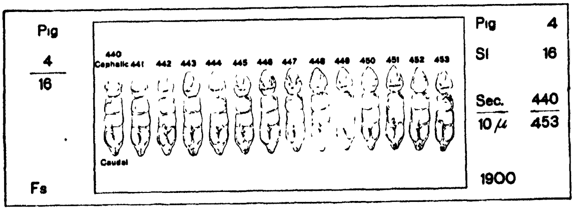


Fig. 271. Frontal Serial Sections Showing the Arrangement and the Numbers of the Sections on This Slide. The Slide is Properly Labeled.

If the sections are too long to mount crosswise, they can be cut apart and mounted lengthwise of the slide, the order being like that of the words in a line of print as with all serial sections.

SAGITTAL SECTIONS

§ **679. Sagittal sections** are those made parallel with the long axis of the body and from the dorsal to the ventral surface, thus dividing the object into equal or unequal right and left (dextral and sinistral) parts.

(1) Imbed the animal or embryo with the right side down in the imbedding mass (§ 628); and arrange the object exactly over the orientation diagram in the bottom of the imbedding box (§ 672).

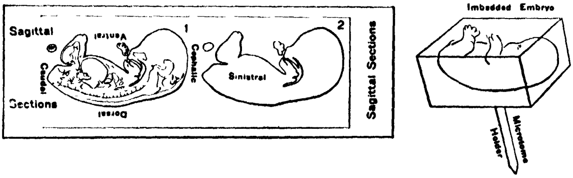


FIG. 272. SERIAL SAGITTAL SECTIONS SHOWING THE POSITION OF THE EMBRYO IN THE IMBEDDING MASS, THE CONNECTION WITH THE MICROTOME HOLDER AND THE ARRANGEMENT OF THE SECTIONS ON THE GLASS SLIDE.

Microtome Holder The metal disc and stem for holding the embryo in position while it is being cut.

Imbedded Embryo The imbedded embryo in the proper position for sagittal sections.

Sagittal Sections A slide of sagittal sections in the proper position on the slide.

1, 2 Serial order in which serial sections are arranged on the slide.

In section *2*, the word sinistral indicates that the left surface of the section faces directly upward. The right side rests upon the glass.

The words cephalic, caudal, dextral and sinistral are inverted under the compound microscope, the sections are reinverted, and will appear like this picture, if the book is turned upside down.

(2) Mount the block of paraffin containing the embryo so that the right side will be next the disc of the microtome holder. The left side will then be cut first, and look up when mounted (fig. 272).

(3) Place in the microtome so that the caudal end will first meet the edge of the knife.

(4) Mount the sections in the order of the print on a page. This

will bring the caudal end to the left, the cephalic at the right, ventral aspect up and dorsal down toward the lower edge of the slide. The dextral face of the section will rest on the slide, and the sinistral face will look up.

Under the microscope the head will be at the left and the dorsal side will appear toward the upper edge of the slide — away from the observer. It will appear like the figure when the book is turned upside down.

If the embryo is large it may be better to turn it around so that the ventral side meets the edge of the section knife. If this is done the sections will have to be cut apart and mounted one by one on the slide, otherwise they would be crosswise of the slide like the frontal sections (fig. 270).

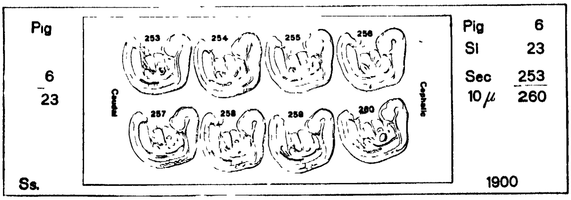


FIG. 273. SLIDE OF SERIAL SAGITTAL SECTIONS SHOWING THE ARRANGEMENT AND LABELING.

§ **680. Labeling serial sections.** — The label of a slide on which serial sections are mounted should contain at least the following:

The name of the embryo and the number of the series; the number of the slide of that series; the thickness of the sections, and the number of the first and last section on the slide; the date. It is also a convenience to have the information repeated in part on the left end (figs. 267–273).

MODELS FROM SERIAL SECTIONS

§ **681. General considerations on modeling.** — Anatomists have for a long time produced models of gross anatomic specimens, and enlarged models for minute details.

Naturally, after serial sections of embryos and organs came to be made with considerable accuracy and of known thickness, there was a desire to make enlarged models which should be exact representations of the original rather than the generalized approximations built up as an artist produces a statue.

Further, the difficulty of getting a true conception of the object by studying only two dimensions in the sections is very great; hence a model giving all three dimensions becomes almost a necessity for the beginner in embryology, and is of enormous advantage to an investigator in working out the true form and relation of complex structures. For modeling a series it is of great advantage to have photographs of the object to be modeled. If possible, the object should be photographed in the fresh state and after fixation. The more aspects photographed, the better.

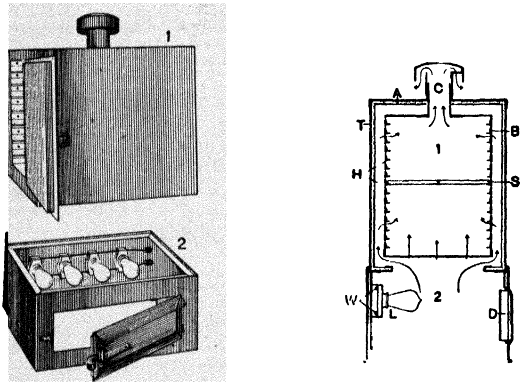


Fig. 274. Drying Oven for Slide Trays.

(From the Anatomical Record).

1 The oven showing all the parts, the oven proper (*1*) is lifted up to show the electric lamps in the base (*2*).

2 Sectional view of the oven (*1*) and base (*2*) showing the construction and the air currents. One tray (*S*) is in position.

A The asbestos lining of the outer shell. *B* One of the numerous ventilating holes. *C* Flue for the escape of air. *H* Runs for the slide trays.

D Door of the support or base (*2*). *W–L* Wiring for the lamps. One can vary the heat by turning out one or more of the incandescent bulbs.

The principles involved in the construction of a model are exceedingly simple: —

1. It is necessary that the embryo or other object to be modeled should be cut into a series of sections of definite thickness.

2. The sheets of modeling material must be as much thicker than the sections as the model is to be larger than the original.

3. The sections must be drawn as much larger than the actual specimen as the model is to be larger than the object.

4. The drawings with the desired outlines must be made directly upon or transferred to the sheets of modeling material which are then cut out, following the lines of the drawing.

5. The different plates of modeling material representing all the sections are then piled up, in order, thus giving an enlarged model of the object with all its parts in proper positon and in true proportions.

MODELS OF WAX

§ **682. Wax models.** — For making wax models, beeswax 820 grams, paraffin 270 grams and resin 25 grams are melted together and thoroughly mixed.

To get the sheets of wax of the proper thickness two methods are available: —

(1) The hot wax is poured into a vessel containing hot water. The wax spreads out into an even layer over the hot water and is allowed to cool. While it is solidifying, it should be cut free from the edges of the vessel. Of course, by calculation and experiment one can put in the right amount of wax to get a plate of a given thickness.

(2) One must have a wax-plate machine consisting of a flat surface — planed cast iron is good — with some means of obtaining raised edges. If these are adjustable by a micrometer screw, it is simple to set them properly for the desired thickness of plate. Then there must be a hot roller. The hot wax is poured on the plate, and with the hot roller resting on the raised edges, the wax is rolled out into a plate. It cools quickly and may be removed for another plate. This is the most rapid and satisfactory method of preparing

the plates. By using a brush with turpentine, the paper with the drawing can be wet and then with the hot roller cemented to the plate before that has been removed from the machine.

The wax plate is cut with a sharp instrument, following the outlines of the object which has been traced upon it by the aid of a camera lucida or the projection microscope. The sections are piled together, some line or lines obtained from a drawing or photograph of the specimen before it was imbedded and sectioned being used as a guide. Finally the whole is welded into one by the use of hot wax or a hot instrument. Models which illustrate complex internal structures are difficult to prepare, but numerous devices will occur to the worker, as the representation of blood vessels and nerves by strings or wires. A large model will need much support which can be given by wire gauze, wires, pins or paper, according to the special needs.

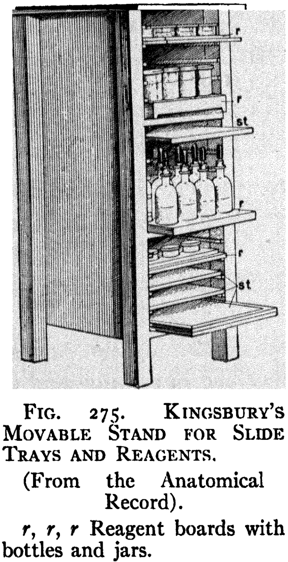


Fig. 275. Kingsbury's Movable Stand for Slide Trays and Reagents.

(From the Anatomical Record).

r, *r*, *r* Reagent boards with bottles and jars.

st, *st* Slide trays.

The stand has furniture slides on the legs and is easily moved on the floor.

A practical method for wax modeling was first published by G. Born, Arch. f. Mikr. Anat., Bd. xxii, 1883, p. 584. The most detailed statements of improvements of the method have been published by Born (Böhn u. Oppel), 1904, and by Dr. F. P. Mall and his assistants. See contributions to the Science of Medicine, pp. 926–1045. Proceedings of the Amer. Assoc. Anatomists, 1901, 14th session (1900), p. 193. A. G. Pohlman, Zeit. wiss. Mikroskopie, Bd. xxiii, 1906, p. 41.

To overcome the difficulty of cutting out the wax plates, Dr. E. L. Mark of Harvard University uses an electrically heated wire moved rapidly by a modified sewing machine (Amer. Acad. Arts and Sciences, March, 1907; Science, vol. xxv, 1907; Anat. Record, April, 1907).

SUSANNA PHELPS GAGE BLOTTING-PAPER MODELS

§ **683. Comparison of wax and paper models.** — Wax has certain inherent defects for models: It is expensive, heavy and fragile. It is easily deformed by the temperature of summer, and the amount of time necessary for the preparation of the plates is great. A wax-plate machine is expensive and bulky.

It therefore seemed worth while to see if there was not some other material obtainable in the open market which would be more suitable and more generally available.

Blotting paper seemed promising, and an actual trial showed it to be admirably adapted for the purpose. Since making the first model in 1905 it has been constantly used in the laboratory of embryology in Cornell University. Models made from it were demonstrated before the Association of American Anatomists in 1905 and before the International Congress of Zoölogy in 1907.

"The advantages of blotting-paper models are the ease and cleanliness of their production and the lightness and durability of the product. The models are broken with difficulty, are easily packed or transported, and when they cleave apart are easily repaired, thus contrasting with the weight and fragility of wax models and their deformation by heat.

"By this process are secured for the original model reconstructed from microscopic sections the same qualities which have made the Auzoux models molded from papier-mâché such useful and lasting additions to laboratory equipment; and, in the hands of Dr. Dwight and Mr. Emerton, of Harvard University, have aided so much in the demonstration of structure and form of special anatomic preparations."

§ **684. Thickness of blotting paper.** — Blotting paper of a uniform thickness of 1 mm. $\frac{9}{10}$ mm., and $\frac{1}{2}$ mm. was found in the market. The 1 mm. is known as 140 lb. A. and costs about two cents for a sheet 61 × 48 centimeters (24 × 19 in.).

The thickness is easily tested by cutting out 50 small pieces, piling them, dipping one end in melted paraffin, and pressing them together. The whole pile should of course measure 50 mm. if the paper is millimeter paper (§ 684a).

§ 684a. — Book-stores, paper dealers and job printers are supplied by the paper manufacturers with samples of blotting paper. One can look these samples over, select and order the kinds desired. The millimeter blotting paper mentioned in the text is one of the cheaper grades, costing by the package of 500 sheets about two cents a sheet (sheets 61 × 48 centimeters, 24 × 19 inches).

§ 685. Size of the model. — In deciding upon the size of the model to be made from a given series of sections one should select the largest section and with the projection microscope throw the image on the table (fig. 276). By using different objectives and different distances from a microscope one can find a size which seems suitable. The magnification may be found by § 409. Then by multiplying the whole number of sections by the thickness of the sections and this by the magnification, one can get the length or height of the model. One must take these preliminary steps and decide upon the magnification to be used or the model is likely to be too large to be manageable or too small to show well the necessary detail.

(1) Suppose the model is to be 100 times the size of the original object, and the object has been cut into a series of sections 10μ thick. Then each section must be represented by a plate or sheet 100 times as long, broad and thick as the object. As the sheets of blotting paper are so large (61 × 48 cm.), one need be solicitous only about the thickness.

As each section is actually 10μ thick and the model is to be 100 times enlarged, the thickness representing each section must be $10\mu \times 100 = 1000\mu$ or 1 millimeter. 1 millimeter blotting paper is used and every section of the series is drawn.

(2) If the blotting paper were only $\frac{9}{10}$ mm. thick, it would be simpler to make the model 90 times the size of the original. If, however, one wished the magnification to be 100, it could be accomplished thus: Each section in the series should be represented by 1 mm. or 1000μ in thickness. But if one uses blotting paper of $\frac{9}{10}$ mm. thickness or 900μ, there is a loss of 100μ for each section and for 9 sections there would be a loss of 900μ or the thickness of a sheet of the blotting paper. To remedy this one uses 10 sheets of blotting paper for 9 sections. This keeps the model in true proportion. In practice each of the sections is drawn upon one sheet

except one of them, and for that two sheets of the blotting paper are united and the sections drawn upon the double sheet.

§ 686. **General rule for the use of blotting paper.** — Divide the thickness by which each section is to be represented in the model by the thickness of one sheet of the blotting paper available. The quotient shows the number of sheets or the fraction of a sheet required for each section.

If a quotient is a mixed number reduce it to a fraction. The numerator represents the number of sheets required and the denominator the number of sections to go with the sheets.

Examples: (*a*) With a series of 10μ sections to be modeled at 100 enlargement each section of the series must be represented in the model by a thickness of $10\mu \times 100 = 1000\mu$ or 1 millimeter. If one uses millimeter or 1000μ paper, then $1000\mu \div 1000\mu = \frac{1}{1}$, and one must use 1 sheet for 1 section.

(*b*) With a series of 10μ sections to be made into a model 100 times enlarged, and with blotting paper of $\frac{9}{10}$ mm. or 900μ thickness, each section must be represented by $10\mu \times 100 = 1000\mu$. If the blotting paper is 900μ thick, then it requires for each section: $1000 \div 900 = 1\frac{1}{9}$ sheets of paper or $\frac{10}{9}$ sheets for one section or 10 sheets for 9 sections, that is, a double sheet for one of the nine sections.

(*c*) With a series cut 15μ, for a 50-fold model, each section is represented by a thickness of $15\mu \times 50 = 750\mu$. If one uses 1 mm. or 1000μ blotting paper, then each section requires $750 \div 1000\mu = \frac{3}{4}$ of a sheet for one or 3 sheets for four sections. In this case one omits every fourth section in drawing, thus: 1st, 2d and 3d sections would be drawn; then the 5th, 6th and 7th; 9th, 10th, 11th, etc., every fourth being omitted.

(*d*) If for the model just considered one had $\frac{9}{10}$ mm. or 900μ paper, then $750 \div 900 = \frac{5}{6}$. That is, there must be 5 sheets of the paper for each 6 sections. In that case every sixth section would be omitted in the drawing, as every fourth section was omitted in (*c*).

It is, of course, best to use sheets of exactly the right thickness to represent the necessary thickness in the model (*a*), but one can produce models with accuracy by duplicating one or more sheets for a group of sections (*b*) or by omitting certain sections of the series in drawing (*c*, *d*).

DRAWINGS FOR MODELS

§ **687.** — The methods given for drawing microscopic preparations in Ch. VIII are all applicable except the free-hand method. This is not applicable, because it is not possible to draw a uniform and accurate enlargement in that way. But the camera lucida method (§ 408) or the projection apparatus method (§ 419) is good. With the perfecting of projection apparatus that method is far the best because one can sit in a comfortable position and use both eyes. It is, indeed, as simple as tracing the outline of actual pictures.

By making negative prints directly on one of the developing papers (§ 488), drawing for models may be wholly avoided.

§ **688. Avoidance of distortion and of inversion.** — In the drawings for models one must, of course, avoid all distortion (§ 402) and the inversion of the image (§ 430). Both these defects are easily avoided if one keeps in mind the optical principles involved, and follows the directions given in Ch. IX.

§ **689. Use of the 6-volt, concentrated filament lamp as a source of light.** — From the experience of the author nothing equals the direct-current arc light for all exacting work in drawing and projection, and for the dark-ground illuminator, but the care required to keep the arc lamp going and to keep the crater centered is so great that the less brilliant light from the 6-volt lamp which requires absolutely no adjustment after being once properly arranged is very acceptable (§ 487). The 6-volt lamp with a transformer is used only on an alternating circuit. As most lighting circuits are now alternating, it is a great advantage; and as this lamp with its transformer can be used anywhere wherever there is an ordinary electric light socket, it is exceedingly convenient. If it is to be used on a direct current circuit, no transformer is used, but the current must be drawn from a storage battery, not from a 110- or a 220-volt circuit from a dynamo.

§ **690. Connections of the transformer.** — If alternating current and a transformer are used, the transformer must be connected to the supply by means of the small connecting wires. The connection with the lamp is by the large terminal wires. Ordinarily the terminals of the transformer are marked so that no mistake need be made. Theoretically the transformer does not modify the energy; it either raises or lowers the voltage or pressure. For the purposes here used the transformer lowers the voltage, and is called a step-down transformer. As the activity or wattage of which the current is capable is not changed by the transformer, and as the wattage is the voltage multiplied by the amperage used, if the voltage is lowered, the amperage is raised proportionally; hence the need of the large wire on the side toward the lamp beyond the transformer where the amperage is increased.

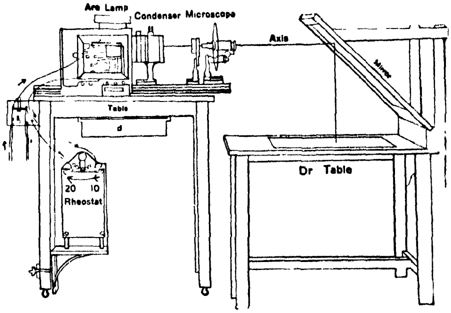


FIG. 276. DRAWING AND PROJECTION OUTFIT WITH LARGE MIRROR ON SEPARATE DRAWING TABLE.

For full explanation see Fig. 181. Instead of the arc lamp here shown the 6-volt incandescent lamp can be used for most purposes (§ 691).

§ **691. Lamp for 6-volt current.** — There are in common use two lamps, one of 72 watts and one of 108 watts. Now as the wattage is the voltage times the amperage, for the 72-watt lamp the amperage with a 6-volt current must be 72 divided by 6 or 12 amperes. For the 108-watt lamp in like manner the amperage is the wattage divided by the voltage, — 108 divided by 6 = 18 amperes. This shows at once why the large wires must be used between the lamp and the transformer. If the usual small wires are used the resistance is too great and part of the energy is used up in heating the wires instead of in heating the filament to supply the light.

§ **692. Arrangement of the lamp for the large projection outfit.** — If the lamp is to be used in the lamp-house instead of an arc lamp for the large projection outfit, it must be centered carefully and put the right distance from the large condenser. The filament takes the place of the crater of the arc lamp and hence should be in the focus of the first element of the condenser, so that the beam between the first and second elements of the condenser will be approximately parallel.

If a two-lens condenser is used, the lamp-filament is slightly within the focus, making the light slightly diverging between the two lenses of the condenser.

A concave mirror or reflector behind the lamp is of considerable advantage, for the light which extends backward is reflected forward to the condenser and is thus available for illuminating the object.

§ **693. Large condenser for drawing.** — If the three-lens condenser is used (fig. 179), and it is much to be preferred, the second element which converges the parallel beam should be of long focus. One of 38 cm. (15 in.) focus has been found very satisfactory. The reason for using the long focus lens is discussed in Ch. IX, § 423, fig. 184.

If a two-lens condenser is used, the second element should also be of longer focus than for ordinary magic lantern work, for the same reason as for the three-lens condenser.

§ **694. Drawing with the small projection outfit.** — If one has no large projection outfit, drawings for models and for publication can be made very satisfactorily with the 6-volt lamp as follows: It is a great advantage to have the lamp in one of the metal lanterns like those used for daylight glass (figs. 46, 53), then scattered light will be avoided. There should be a condenser like that used for the small arc lamp (fig. 78). As the microscope must be horizontal and is ordinarily raised to make the drawing distance 250 mm., the lantern containing the 6-volt lamp must be supported on a box or block to bring the filament of the lamp in the optic axis of the microscope.

When horizontal, the microscope is unstable; hence a weight or better a clamp is put over the feet to hold the microscope firmly so that when once centered it will not move easily. A table with the drawing shelf on the legs is very convenient for getting the desired magnification (fig. 277).

§ **695. Relative position of the lamp and microscope.** — This can be as with the small drawing outfit and arc lamp (fig. 182), or it can be put in line, as with the large outfit. If in line (fig. 179) the mirror is not used, and care must be taken to get all parts lined up to one axis. With the mirror slight deviations from centering can be overcome by inclining the mirror accordingly.

§ **696. Condensers to use with the small outfit.** — For low powers, 50 to 16 mm. (3.2x–10x), the substage condenser of the microscope can be turned aside and the small condenser with the lamp alone employed. In many cases no ocular is used for the sake of the large field. For powers of 8 to 2 mm. (20x–90x) when the ocular is used, it is necessary to use the substage condenser to light with the proper aperture. And if the oil immersion is used, it is a great advantage to make the substage condenser homogeneous immersion also; that is, to have some of the homogeneous immersion fluid between the lower side of the slide and the condenser as well as between the objective and the cover-glass (§ 265).

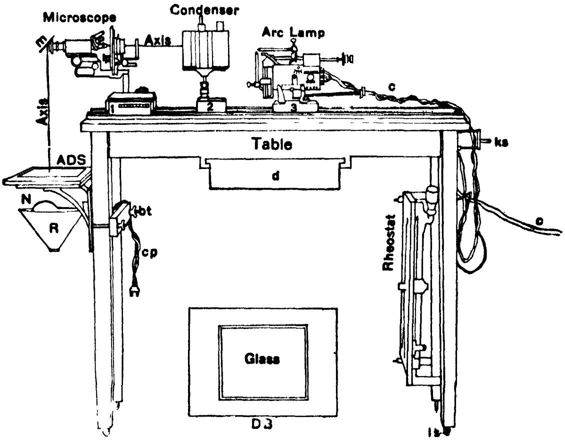


FIG. 277. DRAWING AND PROJECTION OUTFIT.
For full explanation see Fig. 180. For drawing the 6-volt lamp can well take the place of the arc lamp here shown (§ 691).

§ **697. Making the drawings.** — One can draw directly upon blotting paper, but it is so important to have a drawing to refer to that one or more duplicates should be made. This is easily accomplished by putting a sheet of carbon manifolding paper on the blotting paper and a sheet of thin paper over the carbon paper, using thumb-tacks to hold the blotting paper and the duplicating sheets in position.

One should take the precaution to number each drawing as it is made; then confusion in the later processes will be avoided.

§ **698. Cutting out the sheets for the model.** — " With the blotting paper, if the drawings are small, the cutting is easily done with scissors or a knife. When the drawings are large and especially when the model is to be made by representing each section by two or more thicknesses of blotting paper, it has been found that an ordinary sewing machine can be used to do the cutting. By setting the regulator for the shortest stitch, an almost continuous cut is made and the parts are easily separated. If a large sewing-machine needle is sharpened in the form of a chisel, the cut becomes considerably smoother. It has been found advantageous when long continued or heavy work is to be done to attach to the machine an electric sewing-machine motor. Skill in guiding the work is soon acquired. There are some details of a complicated drawing which are more easily cut by the scissors or a knife after the main lines have been cut by the machine."

§ **699. Contrasting colors for marking groups of sections.** — " It is a great advantage in any working model to have sections at regular intervals in marked contrast with the body of the material. Blotting paper of a large variety of colors (black, red, blue, pink) is easily obtained in the market. In the models made every tenth plate was a bright or light color and every one-hundredth was black, rendering rapid numeration easy."

§ **700. Putting the sheets together to make the model.** — " When the paper sections are thus prepared, they are piled and repiled as is usual until the shape conforms to an outline predetermined from photographs, drawings, or measurements made before the specimen was cut.

" It has been found that an easily prepared support and guide for the model in process of setting up is made by cutting the outline to be followed from a block of four or five sheets of blotting paper, marking upon it the lines of direction of every tenth or twentieth section. The colored numerating plates must, of course, conform to the spacing and direction of these lines.

"The preliminary shaping having been accomplished, more exact modeling is undertaken. The paper sections slide very easily upon one another. The most satisfactory means of fastening them together is by the use of ribbon pins, ordinary pins, or wire nails of various sizes, depending on the size of the model. No kind of paste or glue was found suitable for this purpose."

§ **701. Finishing the model.** — "When the model is well formed, inequalities are best removed by rubbing with the edge of a dull knife and smoothing with sandpaper. Any dissections of the model for showing internal structures should be planned for at this stage, for it is now more easily separated than later. It is also at this time that superfluous 'bridges,' which have been left in place to support detached parts, would better be removed.

"To finish the model it is held together firmly and coated with hot paraffin either by a camel's hair brush or by dipping in paraffin and removing the superfluous coating by a hot instrument. One might use a thermo-cautery for this purpose.

"The paraffin renders the model almost of the toughness of wood without destroying the lightness of the paper."

§ **702. Coloring the surface; dissecting the model.** — "For coloring the surface of the model, it was found most desirable to use Japanese bibulous paper, lens paper (§ 54) which had been dipped in water color and dried. Any of the laboratory dyes or inks can be used, such as eosin, picric acid, methylene green, black ink, etc. The colored lens paper molds over the surface with ease and is held in place by painting with hot paraffin. All color and enumeration lines and fine modeling show through the transparent paper.

"When the model ceases to be a working model it can be covered with oil paints mixed with hot paraffin and rubbed to any degree of finish desired.

"One can dissect a model by a hot knife run along the planes of cleavage or cut across them by a saw."

For the literature of blotting-paper models see: Susanna Phelps Gage, Amer. Jour. Anat., vol. v, 1906, p. xxiii; Proceedings of the International Zoölogical Congress for 1907; Anatomical Record, Nov., 1907. (From this paper the above quotations were made.) Zeit. wiss. Mikroskopie, Bd. xxv, 1908, pp. 73–75.

Blotting-paper models have also been made and demonstrated by Dr. J. H. Hathaway and by Dr. J. B. Johnston at the Association of American Anatomists, 1906 (*Proc. Assoc. Amer. Anatomists,* Anat. Record, April 1, 1907); in 1909 by Dr. J. Parsons Schaeffer (Anat. Record, 1910); and in 1916 by Dr. Charles Brookover and Dr. H. Saxon Burr (Anat. Record, 1917).

CHAPTER XIV

MICRO-INCINERATIONS AND THE OPTICAL APPLIANCES FOR THEIR EXAMINATION

§§ 703–730; FIGURES 278–299

INTRODUCTION

Ever since chemistry has given a clue to a method of penetrating into animal and plant composition, there have been ever-increasing efforts made to determine what chemical elements and compounds are present in the different tissues and organs. Because bones and teeth are evidently so largely made up of mineral substances, they were the first structures to be studied to find out exactly what are their mineral constituents. However, the method soon came to be applied to the soft tissues where mineral matter is not so obvious, and these too were found to have a distinct mineral content. Naturally in the beginning the object of study was to determine the total mineral matter without any attempt to give the exact location of the different chemicals found in the tissues. Such general knowledge, important as it is, was not wholly satisfactory, and more and more the aim has been to go beyond the animal or organ as a whole to the individual tissues and cells. And now the investigations are being extended to the constituents of the cells, cell membranes when present, cytoplasm and nucleus. Finally researchers have tried to go still further and determine the chemical constituents of the chromosomes and the mitochondria. (See especially the work of Bensley in the Anat Record, vol. 60, pp. 251–256, 449–455.)

Micro-incineration is for the purpose of locating the different fixed minerals in the structural units of the body. As in ordinary histological procedure, sections or isolations must be made to see the individua tissue elements in multicellular animals and plants, therefore such preparations must be so treated that the organic matter is eliminated and the fixed or non-volatile inorganic matter left in place. As one studies these incinerated preparations, there comes the feeling that if the entire amount of organic matter could be removed from an animal or plant without disturbing unduly the mineral matter, the entire tissue, organ, or animal as a whole would be as recognizable as are the bones under similar conditions.

Among the first attempts to locate the mineral matter in the tissues, the structures were heated red hot to burn off the organic matter, and to the astonishment of the experimenter, the French chemist, Raspail, 1833, the shape of the tissue did not seem changed. (Raspail, in the collateral reading.) While this crude method gave much information, the present refined and successful method of micro-incineration came only when Policard invented the small, regulated electric furnace (Policard, collateral reading). In America the chief exponent of micro-incineration and the results to be attained with it, is Dr. Gordon H. Scott of Washington University, St. Louis. He had the great advantage and privilege of working with Dr. Policard, and learning at first hand the refinements of the method. Dr. Scott has also devised a much improved micro-incinerator (fig. 278) by which any laboratory worker can get excellent results.

§ **703. Chemical constituents of the organism, animal or plant.** — One might fairly expect that owing to the marvelous activities of animals and plants during life they would require some of the most rare and subtle chemical components; but the truth is that the chemical elements found in organic bodies are few in number, only about 20 of the 90 or more already known, and these few are among the commonest, the rarer ones being wholly absent. These elements are: calcium, carbon, chlorine, copper; fluorine; hydrogen; iodine, iron; lithium; magnesium, manganese; nitrogen; oxygen; phosphorus, potassium; silicon, sodium and sulfur. Some others are occasionally found, but they are thought to be accidental or due to the special environment (Starling's Physiology, 7th ed., p. 33).

In the living organism these elements exist mostly in compounds. In incinerated preparations only the fixed or non-volatile compounds remain. (See the references in the collateral reading.)

While a chemist can determine with ease and certainty the composition of the bulk ash in organic matter. Determining the exact character of the chemical constituents in the individual cells has proved a difficult task. The refined methods of chemical microscopy and the use of spectrum analysis and finally of the electron microscope have all been called into requisition with only partial success up to the present time. (See the papers from 1930 to 1940.)

PREPARATIONS FOR MICRO-INCINERATION

§ **704. Fixation.** — It is self-evident that for the determination of the amount and character of the mineral matter in tissues and cells, the preliminary treatment should not add anything nor remove anything of mineral nature. That is, the ideal fixative would render permanent the structural constituents exactly as in life. It would realize in modern histology what the ancients in their mythology ascribed to Medusa of the snaky locks. This ideal fixative has not as yet been discovered. Of the hundreds of combinations which have been tried, not one is universal; all are more or less selective. For example, if one wishes to determine the presence of glycogen, strong alcohol is an excellent fixative, but if lipoid substances are to be sought for, it is very poor.

For the determination of the mineral constituents, the standard fixative is 9 parts of absolute alcohol and 1 part of strong, neutral formalin. Small pieces of tissue or parts of organs are placed in this. Small pieces are used so that the fixer will penetrate quickly and preserve all the cells. Twenty-four or thirty-six hours is usually sufficient. Either a relatively large amount of the fixer is used, or if a smaller amount as compared with the tissue, then it should be changed two or three times for fresh fixer. If one cannot proceed at once with the sectioning, the tissue may remain in absolute alcohol, but it is better to imbed the tissue at once after it is fixed.

§ **705. Imbedding for sectioning.** — The paraffin method is practically always used, as the paraffin is wholly removed in the subsequent steps, and therefore adds nothing to the sections. It is essential for the imbedding that the tissue shall be wholly freed from water. This can be attained by two or more changes of the absolute alcohol.

§ **706. Clearing before paraffin.** — The purpose of this is to remove the alcohol by a liquid which is a solvent of paraffin. It is usually accomplished in two steps: From the absolute alcohol the tissue is passed to a mixture of equal parts of absolute alcohol and xylene for an hour or more, and then it is placed in pure xylene till it appears translucent, or it may be passed from the absolute alcohol to cedar oil and left in the cedar oil till it is translucent. When it is translucent by either of the above methods, the tissue may be transferred to melted paraffin. This is also done in two steps by many workers. The first step is to transfer the cleared tissue to low melting point paraffin (40° to 45° melting point), and after an hour or more in this, it is transferred to melted paraffin of 56° to 58° melting point, and kept in this melted paraffin in an infiltrating oven or box for several hours. It is then fully infiltrated with the hard paraffin and is ready to be put in a block for sectioning. Whatever method is used for finally blocking the tissue for sectioning, it should be remembered that paraffin quickly cooled is more nearly homogeneous, i.e. has finer crystals, than paraffin cooled slowly. The finer the texture of the paraffin the more successful the sectioning.

§ **707. Sections for incineration.** — These must be thin. Rarely will one get good results with sections over 10μ thick, and the usual experience is that sections 3μ, 5μ or 7μ give even better incinerations than thicker ones.

Of course, for such thin, perfect sections the section knife must be sharp, and the microtome a good one. The room temperature should not be over 20° centigrade, and for the thinnest sections a temperature of 12° to 15° c. is more favorable. In his *Plant Histology*, Dr. Chamberlain advises the cool room, and safety razor blades in a suitable holder for sectioning. Many others, including the author, have also found the safety razor blade satisfactory (see §§ 621, 634, and Chamberlain, 5th ed., p. 122).

§ **708. Glass slips for incineration preparations.** — Not all brands of glass slips have been found of sufficiently high melting point to remain undistorted during the incineration which goes up to 600° centigrade or hotter, that is, to red heat. Hence it is wise before wasting time and losing valuable specimens to make sure the glass in the mounting slips will not become distorted by the heating. The author has found the Corex D glass recommended for preparations to be studied under the ultra-violet microscope (§ 308) and Pyrex microscope slips to remain undistorted in every case. If the glass slips are of high enough melting point, it is unnecessary to use sheet platinum to prevent their sticking to the supporting quartz plate.

§ **709. Spreading sections for incineration.** — As nearly all sections, especially thin ones, are more or less wrinkled in sectioning, it is advantageous to flatten or spread them. Some recommend that petrolatum or absolute alcohol be used, but nothing is so satisfactory as the usual water method. A suitable length of ribbon is put on a perfectly clean slide (the Stitt method of cleaning with bon ami §§ 512, 515, has been found satisfactory).

The slide is then warmed on a spreading box or plate (fig. 255–256) and with needles the sections are drawn out flat and arranged. After this the excess water is drained off and the sections are left to dry completely. If they are left over night in a dry, warm place, they will be completely adherent to the slide. It is important to remember that no albumen or other material is to be put on the slide. There is no danger of the sections getting loose during the incineration.

In some cases it has proved advantageous, after the excess water has drained away, to use tissue paper and press the sections down firmly upon the slide with the ball of a finger. The paper is then rolled off the sections by lifting one edge and turning it in a circular manner. If one is skillful, the sections will remain firmly attached to the slide (§ 637).

It is recommended that every other slide of sections be prepared for staining and mounting in the best way experience has shown for the particular tissue. This is important for the stained preparations have the more familiar appearance, and special features are found easily. Of course, for these sections the usual albumen coating on the mounting slide is permissible, but not for the slides to be incinerated.

§ **710. Incinerator and incineration.** — The electric furnace found most satisfactory for micro-incineration is Dr. Scott's modification of Policard's (fig. 278). The heating is regulated by an adjustable rheostat. On a 110–115 volt circuit it reaches 637° C. in 41 minutes if one follows the settings in the table. For the cost of appliances needed for the micro-incineration work see at the end of this chapter.

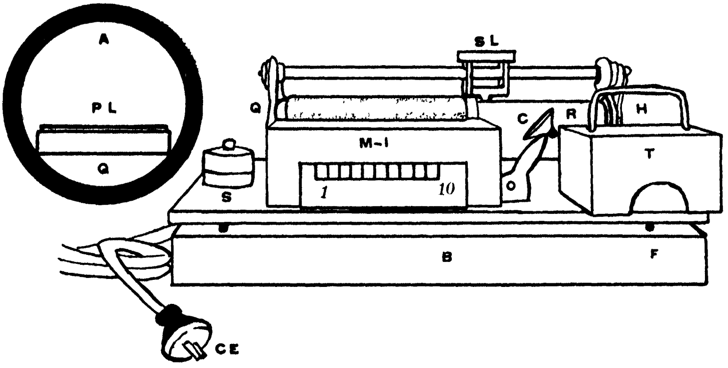


FIG. 278. SCOTT MICRO-INCINERATOR; FRONT VIEW.

A End view of the quartz tube in which the sections are incinerated. It is surrounded by a heating coil.
Q Quartz plate for supporting the slide of sections to be incinerated. The platinum plate (*PL*) shown on its upper face is to prevent slides from sticking. For the corex and pyrex slides this expensive part may be omitted.
B Baseboard of the incinerator; it fits the laboratory lockers.
C Cap or lid to cover one end of the quartz tube.
CE Cap for connecting with the electric circuit.
F Rubber support between the insulating plate and the base.
H Handle of the insulating cover of the quartz tube.
M-I Insulating block containing the quartz tube.
Q Quartz tube; it is surrounded by a heating coil and is imbedded in insulating material.
R Variable rheostat with its rider (*SL*).
SL Rider on the adjustable rheostat to vary the resistance.
T Top of the insulating material covering the quartz tube. It is shown lifted off to hasten the cooling of the quartz tube after an incineration is completed.
1–10 Scale of 10 centimeters to show the amount of reduction.

The furnace is designed to be connected to the usual house lighting circuit. As stated in the legend of fig. 278, there is associated with it an adjustable rheostat by which the heating is gradually increased. This is important, for if heated too rapidly, the ashes do not remain in position.

When ready to incinerate a slide of sections, place the slide on the quartz plate and push the plate bearing the sections into the heating tube to its middle. This tube is long enough to admit two slides at once, but the incineration is usually more successful if but a single slide is placed in the middle.

For sections where no mercury was used in the fixation, the paraffin need not be removed from the sections. The heat in incineration will burn it off. (See also § 712 for mercury fixatives.)

While incinerating, it is found in practice that an interval timer is convenient to mark the different steps, then one can attend to other duties during the intervals.

§ **711. Time of incineration.** — There is considerable difference in the ease of burning off the organic matter with different specimens. As there has not yet been sufficient experience to standardize the method for all objects, the individual worker must do considerable experimenting with his particular material.

In general, it takes about 41 minutes for a successful incineration when the schedule in the table is followed. In a dark or dimly lighted room the quartz tube will show a red heat over its entire length at the 7th or 8th interval. The current is then turned off and the cap *C* pulled back to allow free access of air. The insulating top *T* is lifted off to hasten the cooling.

The following table shows the different settings of the rider (*SL*) on the resistance coil (*R*) for each of the eight intervals and the time of each interval. It also shows the amperes, volts of the line, the watts used, the millivolts when the incinerator is in the circuit, and finally, the temperature centigrade at the end of each setting.

With some tissues a slower increase in heating and a longer time than given in the table give better results.

It is seen from the table that it takes 25 minutes for the incinerator to increase from room temperature of about 20° to 300° C. and an additional 15 minutes to increase the temperature to 600° C. With the current turned off, the insulating top removed and the cap turned back from the quartz tube, it takes 30 to 40 minutes for the furnace to cool down to 300°, when it is safe to remove the incinerated slide with forceps. The slide will soon cool in the free air. If to the naked eye the ashes are gray or white and have the form of the incinerated sections, the incineration is usually successful.

Sometimes the ashes look confused or brown. This is likely to happen if the sections are too thick or not completely dry before the incineration begins. Like other operations in microscopy, micro-incinerations require much exactness in detail, and even then the results are sometimes disappointing. When success is obtained, however, one feels well paid for all the trouble.

For the calibration of the electric furnace shown in figure 278 a Chromel-Alumel thermocouple and a potentiometer with the necessary ammeter and voltmeter were employed and the observations made in the laboratory with the alternating electric current available where the incinerations were to take place.

Table Showing the Calibration of the Micro-Incinerator.

Setting	Minutes	Amperes	Volts	Watts (AxV)	Millivolts	Temperature, ° C.
1	10	1.60	113	180.80	4.32	104
2	5	1.90	113	214.70	6.29	150
3	5	2.18	113	246.34	9.68	235
4	5	2.50	113	282.54	12.80	311
5	5	2.82	113	318.66	16.20	394
6	5	3.25	113	367.25	20.40	492
7	5	3.70	113	418.10	25.00	601
8	1	3.82	113	431.66	26.50	637

§ **712. Further methods of fixation for incineration.** — As stated, it is axiomatic that ideally the fixer should not add anything or remove anything from the tissue to be incinerated. But as every worker with the incineration method soon realizes, alcohol-formalin preparations are far from ideal histologically, and some tissues become so hard that they can scarcely be cut (e.g., ligamentum nuchæ).

One of the simplest and best fixers that have been used for a great variety of tissues is a mixture of a 3% aqueous dichromate of potash solution to which has been added neutral formalin in the proportion of 90 cc. dichromate solution, 10 cc. strong, neutral formalin. Small pieces are fixed in this two or three days, changing the freshly prepared mixture each day. Then it is washed several hours in running water, transferred to 67% alcohol for one or two days, then 82% alcohol until one is ready to proceed with the imbedding. The dehydration should be thorough in 95% and absolute alcohol. The clearing is then by means of absolute alcohol and xylene, then pure xylene or cedar oil. Infiltration is accomplished by low-melting and then high-melting paraffin as in § 706. With these sections the paraffin need not be removed. If a fixer with mercury, such as Zenker's fluid or Helly's fluid, is used, then the paraffin must be removed and the mercury got rid of by soaking in iodin. (See § 597.) After the alcohol used to remove the iodin, the sections are allowed to dry in the air, and may then be incinerated. In any of these methods only small traces of chromium and potassium salts are added, but the amount is only a trace, and the general result is vastly superior to the alcohol formalin method both for the histological and for the incineration appearance as one can see by comparing preparations made by the different methods.

§ **713. Preservation of incinerated sections.** — It cannot be too highly emphasized that the ashes of the incinerated specimens are very delicate and can be easily disarranged if brushed or the fingers put upon them. If one is careful the uncovered preparations can be examined, but to avoid injury it is far safer to put a cover-glass over them at once. To do this a clean cover is placed over the specimen and held in place at one end by the thumb and finger. Then with a hot wire in the other hand a seal of beeswax is run along the four edges. The best way is to get the wire quite hot in a bunsen flame and then press the wire against a mass of beeswax to get it well coated. Then it is run along the edges. The beeswax cools almost instantly and makes a good seal which can later be covered with shellac cement for added strength and permanence. Many advocate the use of paraffin for the seal, but the high melting point of the beeswax makes it more suitable for the purpose, and less likely to melt and run under the cover and spoil the preparation.

One can also make a shallow cell of shellac or balsam or other cement about the ashes; and when nearly dry the slide can be warmed and the cover pressed down all around against the cement till it adheres The beeswax method is preferable, however.

§ **714. Mounting medium for incinerations.** — As described above incinerated preparations are mounted in air. This is the most satis factory way, for any mounting medium so far suggested is likely to disarrange the ashes or to obscure the finer details. It is instructive, however, to be able to compare the ashes in air and in some mounting medium. This is easily accomplished if the slide has upon it several sections of a ribbon. Before adding the cover-glass a small drop of petrolatum (§§ 536, 602) is put upon one of the end sections. If the cover is then added as directed, the mineral oil will spread over one or two of the sections, the remainder being in air. Then it is easy to compare the ashes in the air with those in the petrolatum.

§ **715. The mineral matter of plant tissues.** — Judging by the literature, much less work has been done in incinerating plant tissue than animal, but as stated above, it was plant tissue that Raspail found so interesting when the organic matter was burned away. In the limited experiments carried on in the Cornell laboratory, the plants lent themselves as readily as the animals to this form of investigation. The presence of much silica in many cases adds to the striking appearance of the ashes. For example, the teeth or serrations along the edges of grass leaves are almost completely unchanged and have the same clear outlines as in the stained preparation, and as shown later the mineral remains of plant tissues polarize almost as strongly after incineration as before, thus being in strong contrast to the mineral matter of animal tissues.

In preparing the tissues for incineration one must take the same precautions as for animal tissues (§§ 704–712).

§ **716. Minerals in pathological material.** — The incineration method has been utilized with informing results for pathological tissues. As one might expect from arteriosclerosis in the blood vessels, the mineral contents in pathological material is often considerably in excess of that in normal tissue.

OPTICAL APPLIANCES FOR THE STUDY OF MICRO-INCINERATIONS

§ **717. Dark-field microscopy.** — From the nature of the material, a dark-field is almost a necessity for the study of the ash after micro-incineration. Fortunately this study can be made most successfully with the rather simple apparatus found in every laboratory. For the general understanding of dark-field microscopy the reader is urged to go carefully over the discussion of the dark-field microscope in Ch. III.

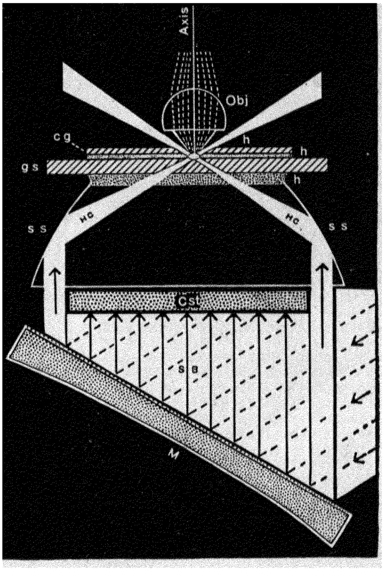


FIG. 279. PARABOLOID DARK-FIELD CONDENSER.

SB Solid beam of light from the mirror (*M*) to the central stop, which allows only a hollow beam to pass on to the condenser.

Cst Central stop to intercept all but the border rays.

HC Hollow cone passing on to illuminate the object.

ss Silvered outside surface of the paraboloid.

h, h, h Homogeneous media below the slide, covering the object and above the cover-glass.

cg, gs Cover-glass and glass slide.

Obj The first element of the objective showing the light deflected from the object by dotted lines.

For the special work with incinerations, the following observations upon their illumination are added after much experience.

Micro-incinerations are most satisfactorily studied with moderate powers, therefore rather small apertures and large fields are utilized.

The proper illumination may be most easily obtained by the use of the ordinary condenser and a central stop (§ 719).

Furthermore, while much of the ash is in optical contact with the glass slide, the overlying mineral substance is in air. This makes the ordinary refracting condensers now found on nearly all laboratory microscopes entirely adequate. It is well to recall that before Wenham

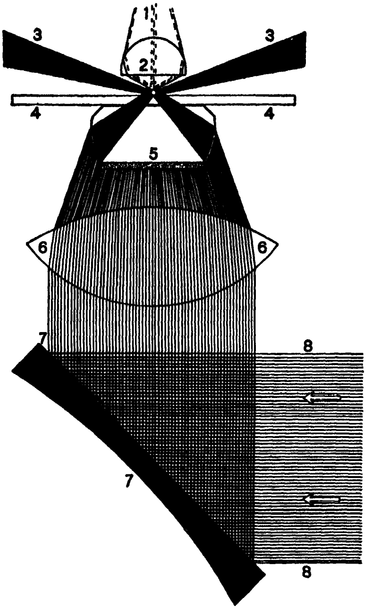


FIG. 280. REFRACTING CONDENSER WITH A FIXED DARK-STOP BELOW THE UPPER ELEMENT.

1 Rays of light from the object in the focus of the dark-field condenser.
2 Front lens of the objective.
3–3 Sectional view of the hollow cone from the condenser. It lights the object at its focus, and is of greater aperture than the objective. (See figs. 294–296.)
4–4 Glass slide supporting the object. It should be of a thickness to bring the object at the focus of the hollow cone, and should be in immersion contact with the top of the condenser.
5 Numeral placed just above the dark-stop, which eliminates the central part of the light cone.
6–6 First or lower element of the condenser. Most often the dark-stop is below this element. (See fig. 279)
7–7 The plane and concave faces of the mirror.
8–8 Parallel rays from the light source.

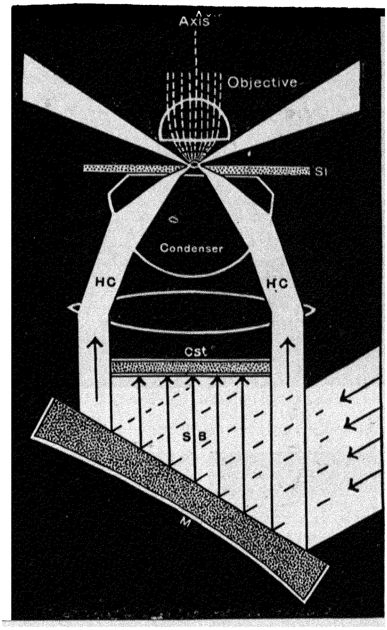


FIG. 281. REFRACTING CONDENSER WITH CENTRAL STOP BELOW TO FORM A HOLLOW CONE OF LIGHT FOR DARK-FIELD ILLUMINATION.

SB Solid beam of light from the mirror (*M*) to the central stop (*Cst*), which cuts out all but the border rays.

HC Hollow cone focusing on the object on the slide (*Sl*). Compare with figure 70 where only the border rays seem to be extending from the mirror to the condenser. This is the usual method of representing all dark-field condensers.

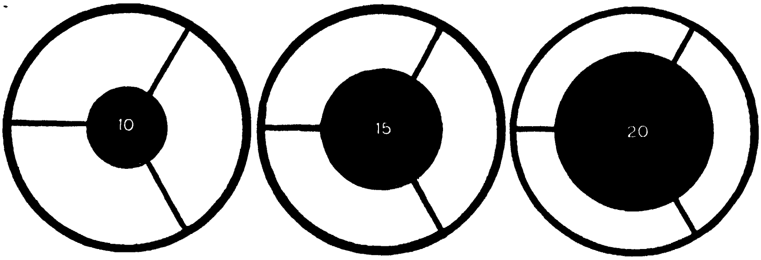
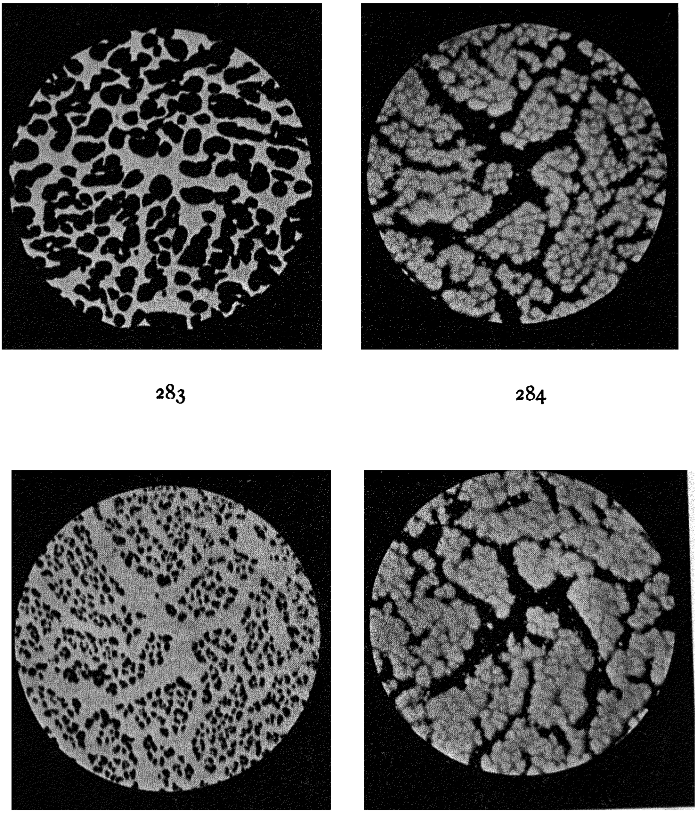


FIG. 282. CENTRAL DARK-STOPS OF 10, 15 AND 20 MILLIMETERS TO USE WITH REFRACTING CONDENSERS AND OBJECTIVES OF DIFFERENT POWERS.



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Figs. 283–286. Transections of the Ligamentum Nuchae of the Ox.
(All at a magnification of 250.)

283 Specimen stained with Verhoeff's hematoxylin. Bright-field photomicrograph.
284 Unstained specimen incinerated to show the mineral matter. Dark-field photograph.
285 The same specimen photographed with a light-field.
286 The same specimen photographed with a dark-field.

(in 1850–1856) introduced the paraboloid condenser (fig. 281) for high power dark-field illumination, the English microscopists were making much use of the refracting condensers for dark-field lighting by inserting an opaque central stop below the condenser to eliminate the central part of the light cone, and thus light the object by rays at such great obliquity that none of them could enter the objective, hence the objects seemed to shine by their own light in a dark field. The refracting condensers also light a relatively large field, and the specimens need not be on a slide of such definite thickness as is required by the special dark-field condensers.

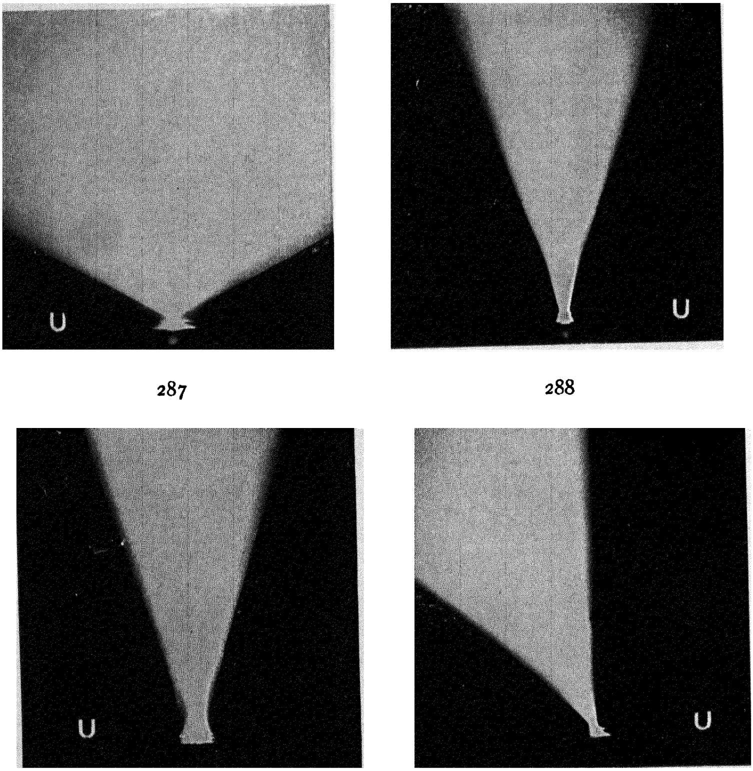
§ **718. Relative numerical aperture of condenser and objective for dark-field illumination.** — By glancing at figures 291–292 and 294–296, it will be seen that the aperture of the objective must be considerably less than that of the condenser or the rays of light from the condenser will enter the objective and render the field light. This involves two requirements: There must be some means (1) of varying the size of the dark-stop under the condenser and (2) of varying the aperture of the objective by means of a reducing diaphragm, most conveniently of the iris type in the objective.

Generally speaking, an aperture of less than 0.65 N.A. is most successful in objectives to be used with refracting condensers.

Table of the diameter of the central dark-stop below the condenser of 1.20 or 1.40 N.A. and the aperture of objectives to give the best effects with incineration specimens.

Objective	Full N.A.	Best N.A. for Incinerations	Size of Sub-stage Stop
16 mm.	0.25	0.25	10 mm.
8 mm.	0.50	0.40–0.50	10–15 mm.
4 mm.	0.66	0.45–0.60	15–20 mm.
3 mm.	0.85	0.50–0.60	20 mm.
1.8 mm. im.	1.25	0.50–0.60	20 mm.

In figures 294–296 is shown the central part of the light cone eliminated by the different central stops of 10 mm., 15 mm. and 20 mm. diameter, and by the dotted lines is shown the angle of the dark center which is utilized by the different objectives for dark-field observation. These diagrams show convincingly that the dark area in the cone cannot all be utilized for dark-field illumination by the objective. Apparently the diffracted light along the edges of the hollow cone is sufficient to give a light halo around the margin of the dark field if the aperture of the objective is too great. The diagrams show also the thickness of the hollow cone of light remaining



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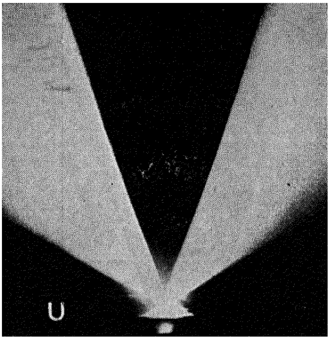
289 290

FIGS. 287–292. CONES OF LIGHT IN URANIUM GLASS IN IMMERSION CONTACT WITH THE TOP OF A CONDENSER RATED AT 1.40 N.A.

U Uranium glass with refractive index n_D 1.5069.
287 Cone of light with full aperture. Plane mirror.
288 Cone of light with substage iris open 10 mm. Plane mirror.
289 Cone of light with iris open 10 mm. Concave mirror.
290 Cone of light with iris of 10 mm. Plane mirror tipped.



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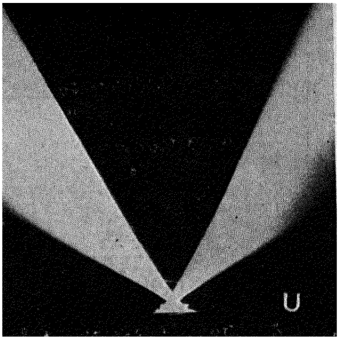

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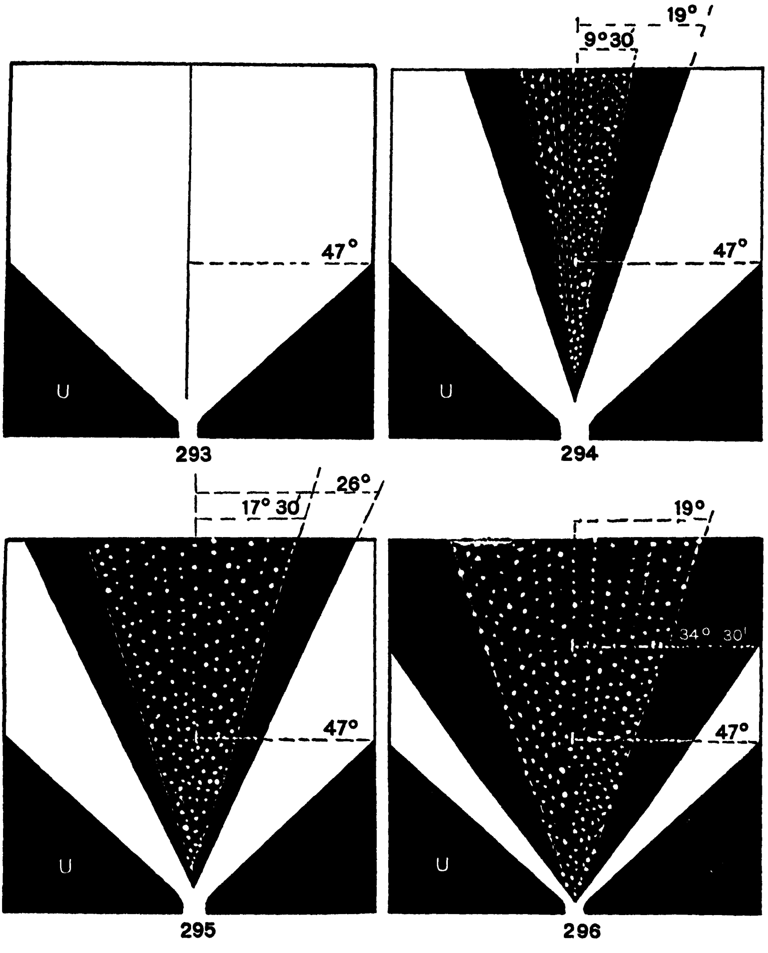
291 Hollow cone with a 10 mm. substage dark-stop. Plane mirror. Iris of condenser wide open.

292 Hollow cone with a 20 mm. dark-stop. Plane mirror. Iris of condenser wide open.

By comparing fig. 287 with 293, and 291–292 with 295–296 it will be seen that the greater the aperture of the condenser the thicker will be the hollow cone of light with a given dark-stop and consequently the greater the amount of light to illuminate the object at its focus.

with the different substage stops. Of course the thicker the hollow cone of light, the greater is the amount of light available for illuminating the object at its focus. It is also seen, except for the 16 mm. objective, that the available angle is less than the numerical aperture of the objective used.

§ **719. Best dark-field effects.** — For obtaining the best dark-field effects two great principles must be kept constantly in mind: (1) That the illumination must be of sufficient intensity to render the finest details of the object visible, and (2) That the aperture of the objective must be great enough to resolve the visible particles, i.e., to show the details (§ 264). One can see an unforgettable demonstration of these principles as follows: Get by trial the most favorable illumination and the best aperture of the objective to give the clearest view of the details, then keeping some fine dot or other detail in sight, gradually dim the light by putting neutral glasses or ground glasses in the path of the light source. As the light is dimmed the fine details are lost. Then restore the light to give the clearest image. Now gradually close



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Figs. 293–296. Diagrams to Show the Cones of Light in Uranium Glass (U) from a Condenser Rated at 1.20 N.A. The Dotted Portion Shows the Aperture Available for Dark-Field.

U Uranium glass in homogeneous contact with the top of the condenser. It is fluorescent and has a refractive index of n_D 1.5069.

293 Cone of light with the substage iris wide open. As indicated u, or half the angular aperture, = 47°. It is the same in all.

294 Hollow cone with a 10 mm. dark-stop. The dark hollow has u, 19°, but only 9° 30′ of this is available for the best dark-field effects.

295 Hollow cone with a 15 mm. dark-stop. This gives a dark hollow of u 26°, but only u 17° 30′ gives the best effects.

296 Hollow cone with a 20 mm. dark-stop. The dark-center is u 34° 30′ of which only about u 19° is available for the best dark-field effects.

The smaller the dark-stop the more the light for illumination, but the smaller must be the aperture of the objective.

the iris in the objective and soon the aperture will become too small for resolving the fine details, and they will disappear. On increasing the aperture, they will reappear.

It must not be forgotten too, that for the best dark-field effects with all forms of condensers the slide must be in immersion contact with the top of the condenser, otherwise only an aperture of 1.00 N.A. can pass into the slide to illuminate the object on its upper face. As many of the particles are in optical contact with the slide, the rays of an aperture greater than 1.00 are very important for illuminating the object. (See fig. 73 and § 190.)

§ **720. Determination of the aperture of the objective.** — For the information given in the above table (§ 718), the best light and the most favorable aperture of the objective was found by trial in each case, then the aperture of the objective actually used was determined by removing the objective with care not to change the objective iris, and employing the apertometer. (See for using the apertometer § 266.)

§ **721. Change from dark-field to bright-field illumination; combining bright- and dark-field illumination.** — The refracting condensers have a great advantage over the special dark-field condensers in that with them it is easy, without disturbing the preparation in the least, to change the illumination or to combine the dark- and the bright-field lighting. If the central stop is in place, the field will be dark, but if that is removed and the substage iris is used, the field will be light.

Furthermore, if one wishes to see the effect of a combination of light-field and dark-field, that is also readily accomplished as follows: A small central dark-stop is used, say one of 5, 7, or 10 mm. which will eliminate only a small central cone. For example, one might use a 10 mm. dark-stop and the 3 mm. or the 4 mm. objective. If the iris of the objective is closed sufficiently, there will be a dark-field; but if it is opened it will include in the aperture of the objective some of the edge rays of the hollow cone. The central stop gives the dark-field illumination and the edge rays of the hollow cone the bright-field illumination.

If, when the iris is closed sufficiently to give a dark-field one looks into the microscope and gradually opens the objective iris, there will first appear a bright halo all around the field. This will gradually spread over the whole field as the iris is opened. It is instructive also to reverse the process and attain a dark-field again.

§ **722. Comparison of stained and incinerated tissues.** — As recommended above, every other slide of a ribbon is mounted for staining. With the thin sections the tissue on the stained slide and that on the incinerated one are nearly identical so that one can see the histological elements in the stained sections, and the same elements represented by the mineral matter in the incinerated ones. It is of advantage to have two microscopes near together, the one for the stained preparation lighted with the bright-field and the incinerated one with dark-field illumination. One can look from one to the other and make sure that the same elements are being studied. If one has a comparison ocular (fig. 142) one can see the two fields at the same time and thus make the comparison more exact. Two wholly different microscopes answer very well, however. By repeated comparison, one soon learns to detect special structures by the ash in the incinerated specimens with the same certainty as with stained specimens.

§ **723. Dark- and bright-field appearances.** — In the accompanying photomicrographs (figs. 283–286), the appearances are strikingly different for the same tissue, depending upon the method of preparation and also upon the method of illumination. Figures 283 and 285 were photographed with the bright-field microscope, while figures 284 and 286 were made with a dark-field microscope. Figures 284, 285 and 286 are of identical parts of the same specimen. The stained specimen (fig. 283) is of the same elastic tissue, but could not be quite identical with the other figures. The cut ends of the fibers were stained black by Verhoeff's method, and are markedly larger than the mineral matter in each fiber although all were magnified exactly the same (250 diameters). In the stained specimen there seems to be empty space between the black fibers, but this is not the case. The white spaces are filled with collagenous connective tissue as can be seen in a specimen stained with Mallory's connective tissue stain, which colors the elastic tissue red and the ordinary connective tissue blue. In unstained preparations under the dark-field, also in a preparation like this, the dark-field illumination reveals the collagenous tissue; and with the ultra-violet microscope the elastic tissue in unstained sections mounted in petrolatum fluoresces blue-white, but the intervening collagenous tissue does not fluoresce and therefore the area between the elastic fibers appears black. On the other hand, with the dark-field microscope the elastic fibers are dark and the collagenous tissue a brilliant white (fig. 130, A B). These observations will also emphasize the necessity of using many methods if one is to gain a true insight into the real complexity of organic structure.

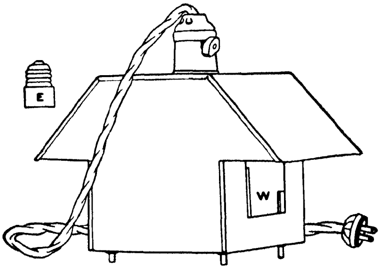


FIG. 297. CHALET MICROSCOPE LAMP.
(See figs. 46–47.)

LAMPS FOR ILLUMINATION

§ **724. Intensity and visibility.** — As stated above (§ 719) there must be sufficient intensity of illumination to render visible the objects one wishes to see. For this with all powers, both for light- and for dark-field study, one of the research lamps (figs. 298–299) answers well. For bright-field observation with all powers, and for the lower powers in dark-field observation the Chalet Lamp (fig. 297) is adequate.

For both lamps the plane mirror is usually the one to employ, and, if dark-field illumination is desired, the substage iris diaphragm is opened fully (figs. 287, 293). As seen by fig. 288, if the substage iris is partly closed, the aperture of the condenser is lessened and a dark central stop would eliminate all the light.

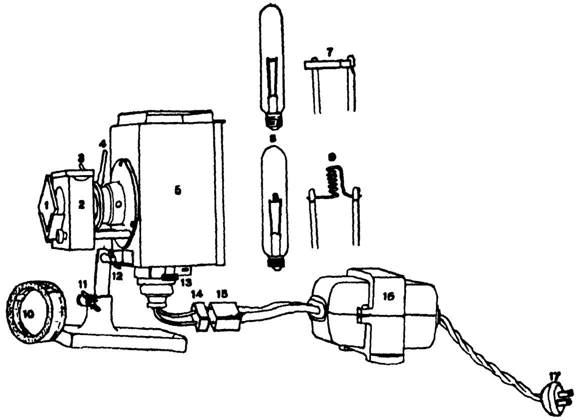


FIG. 298. RESEARCH MICROSCOPE LAMP WITH 108-WATT 6-VOLT BULB AND ACCESSORIES.

(For full description see fig. 80.)

In figure 289 the concave mirror is used, and in 290, the light is oblique. Figures 291–292, 294–296 show that with the substage iris wide open, a dark, central stop leaves a rather thick shell or hollow cone to light the object at its focus.

The research lamps are too brilliant for some specimens. The light can be softened as desired by introducing neutral tint glasses or ground glasses in the path of the beam.

While the lighting recommended above gives the best results, it is quite marvelous how much can be seen with lights of lower intensity. This was demonstrated to the author on one occasion when snow and floods eliminated the electric lights. Then a kerosene lamp, a naked candle flame, and an electric flash light were tried out of curiosity. The results were astonishingly good for both bright- and dark-field observation with objectives as high as a 4 mm. Such experiences give one an inkling that the old histologists were not so badly handicapped as is sometimes thought, and the insight they gained into histological structure with their simple appliances is not quite so astonishing.

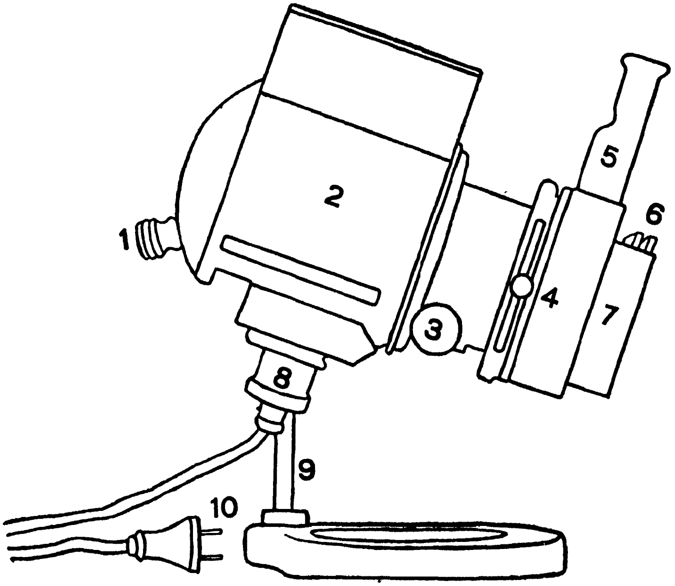


Fig. 299. Research Microscope Lamp of the Spencer Lens Company.

1 Knob with which to incline the lamp-house when it is hot.
2 Lamp-house.
3 Screw head for focusing the condenser.
4 Handle of the iris diaphragm in front of the condenser; the 4 is on the water-cell container.
5 Pyrex water-cell partly raised in its holder.
6 Neutral-tint glasses in the color-screen holder.
7 Holder for neutral tint and color screens.
8 Lamp-socket.
9 Lamp-house standard on which it can be raised and lowered.
10 Base of the lamp-house support and electric plug cap.

§ 725. Micro-incinerations under the polarizing microscope. — The micro-incinerations of animal tissues with the abundant mineral matter after incineration must be in an amorphous condition as usually there is no double-refraction shown under the crossed nicols of the polarizing microscope. With plant tissues, however, the polarization after the incineration was almost as good as before. The serrations along the edge of grass blades were apparently quite unchanged by the incinerating heat both in form and in reaction in polarized light. This striking difference in the minerals left by incineration of animal and plant tissues was quite unexpected, but was present in all the cases examined except in arteriosclerosis.

§ **726. Micro-incinerations in ultra-violet radiation.** — In both animal and plant tissues the ashes show no fluorescence in the numerous examples tested. Comparison specimens unstained and unincinerated and mounted in petrolatum after the removal of the paraffin by xylene, gave brilliant fluorescence in both animal and plant tissues.

In the ultra-violet, then, the animal and plant tissues agree, but the ashes are ordinarily in striking disagreement in polarized light.

Special Apparatus Needed for Micro-Incineration Investigation

§ **727. Policard Incinerator Modified by Scott.** — This is shown in figure 278 with its regulating rheostat. The cost is approximately $40–$50.

§ **728. Optical appliances.** — It is assumed that the laboratory or private worker already has a good laboratory microscope with a refracting, substage condenser, and low-power objectives up to 16 mm., also a good lamp for use with bright-field.

Objectives needed and desirable: It will be noted by comparing the price here given with that in the manufacturer's catalogues that it costs $5.00 additional in each case to have the iris diaphragm present.

Objectives:	8 mm. achromatic, N.A. 0.50, iris	$20.00
	4 mm. achromatic, N.A. 0.66, iris	$22.00
	3 mm. achromatic, N.A. 0.85, iris	$25.00
	1.8 mm. oil immersion, N.A. 1.25, iris	$40.00

For most work with incinerations the 16 mm., and the 8 and 4 mm. objectives with iris are sufficient. If one wishes to carry the investigation as far as possible, the 3 mm. dry, and the 1.8 mm. oil immersion with iris will be needed. It is also desirable to possess one of the special, dark-field condensers of the paraboloid or cardioid type.

For the refracting condensers, substage dark-stops of 10 mm., 15 mm. and 20 mm. are needed. One cân make them if necessary. (See § 180.)

§729. **Research microscope lamp** (**figs. 298–299**). — The cost is about $60.00. For photomicrographs and for dark-field work the type with 6-volt 108-watt lamp bulbs requiring a step-down transformer is much to be preferred to any other type. (See pp. 146–150.)

The lamps and the objectives here recommended may be obtained from the Bausch & Lomb Optical Company, Rochester, N. Y., or from the Spencer Lens Company, Buffalo, N. Y.

§ 730. **Uranium glass for showing the form and path of light beams.** — For teaching purposes and for the individual worker it is of great advantage to see exactly how the light from the condenser actually appears with different arrangements. The pictures shown in figures 287–292 represent some of the appearances. As described in the legend of those pictures, they were obtained by placing a plate of uranium glass of refractive index n_D 1.5069 in homogeneous contact with the upper face of the condenser. Because of its fluorescent character one can see in this glass just how the cone of light from the condenser appears with the plane and the concave mirror, with the light central or oblique, with the full aperture and with the aperture reduced by the substage iris, or by a dark-stop to cut out the central part of the light cone. After experimenting with this help one will always have a clear conception of just what happens to the light under different conditions.

A plate of uranium glass 50 mm. square and 12 to 18 mm. thick with all the faces polished costs	$5.00
A cube with 25 mm. sides all polished also costs	$5.00

This glass is called fluorescent canary, and may be had of the Corning Glass Works, Corning, N. Y.

The microscope slips of high melting point required in § 708 which will not soften and become distorted in the incineration process may be had of the Corning Glass Works also.

That these high melting-point slips may not be confused with the ordinary glass slips used in microscopy, the author has found the size of 25 × 65 mm. used with the ultra-violet microscope satisfactory and convenient (see § 308, and figs. 218, 224).

The cost per 100 with cut edges (i.e., not ground) is approximately $6.00

COLLATERAL READING FOR CHAPTER XIV

RASPAIL, FRANÇOIS-VINCENT. 1833. Nouveau système de chimie organique fondé sur des méthodes nouvelles d'observation. p. 528, *et seq.* Raspail has been claimed as the founder of chemical microscopy or microchemistry. He applied the microscope to the study of plant and animal structures with great success, and among his methods was that of incineration to show the presence of mineral matter in soft tissues after the organic matter had been burned away.

——. 1838. Same, 2d edition in three volumes.

LIESEGANG, R. E. 1910. Die Veraschung von Mikrotomschnitten. Biochem. Zeitschr., Vol. 28, p. 413. He used sections 20μ thick and heated over a Bunsen flame. Such sections do not give good results at present.

POLICARD, A. 1923. Sur une méthode de micro-incinération applicable aux récherches histochimiques. Bull. Soc. Chim. de France, 4th ser., 33, 1551.

——. 1923. La minéralization des coupes histologiques par calcination et son intérêt comme méthode histochimiques générale. Compt. Rend. Acad. Sci, 176, 1012. In these first papers Policard describes his incinerator and gives explicit directions for the procedure in micro-incineration.

——. 1932. Some new methods in histochemistry. The Harvey Lectures delivered under the auspices of the Harvey Society of New York, 1931–32, under the patronage of the New York Academy of Medicine, 204–26. In this lecture Policard describes the micro-incineration process, but points out that it is only the first step in the analysis of the minerals present.

POLICARD A., AND OKKELS, H. 1930. Localizing inorganic substances in microscopic sections. The Micro-Incineration Method. Anat. Record, 44, 349–61. This paper contains a picture of Dr. Policard's micro-incinerator and some excellent photomicrographs of incinerated specimens with a good discussion of the process and the findings. There is also an extended bibliography.

POLICARD, A. 1938. La Méthode de la Microincinération, exposé pratique. Actualités Scientifiques et Industrielles 765. Histophysiologie.

SCOTT, GORDON H. 1933. A critical study and review of the method of micro-incineration. Protoplasma, 20, 133–51. This paper is full of useful information concerning the history and method of incineration, and contains 74 references in all fields where micro-incineration has been applied.

——. 1933. The localization of mineral salts in cells of some mammalian tissues by micro-incineration. Amer. J. Anat., 53, 243–79. This paper contains 46 figures of micro-incinerations in three plates and gives references to 46 other papers, including those of special historical interest.

GAGE, S. H. 1938. Apparatus and methods for micro-incineration. Stain Technology, Vol. 13, pp. 25–36.

HINTZSCHE, ERICH. 1938. Das Aschenbild tierscher Gewebe und Organe. Ergebnisse der Anatomie und Entwicklungsgeschichte. 32 Band, pp. 63–136. This monograph gives a general review, and refers to 208 other papers.

UBER, FRED. 1940. Microincineration and Ash Analysis. This paper deals especially with plant tissues. Botanical Review, Vol. 6, pp. 204–226.

CHAPTER XV

BRIEF HISTORY OF LENSES AND MICROSCOPES

FIGURES 300-313

Lenses. It is difficult to think of a world without lenses. All apparatus like the moving picture machine, magic lantern, photographic camera, the microscope and telescope and spectacles, would be no more. But it is not to be forgotten that the most splendid creations in the world of art, as those of the Greeks; and in the world of literature, as those of the Hebrews, the Greeks and the Romans; the architecture of the Orient, of Egypt, Greece and Rome; and the feats of engineering of the ancient world were all independent of lenses and the optical instruments which they make possible. But what immeasurably greater insight into the real world has come with these "optic glasses"! What revelations as to the cause of disease, the structure of the universe in its smallest details by the microscope, and in its larger ranges by the telescope; and greatest of all for the common man, has come the power, by means of spectacles, to make good use of the years that hygiene has added to the average human life.

That nature made lenses during every rain-storm and every heavy dew and in the tears of every gum and balsam tree, we know now; and for the almost infinite years which man has been upon the earth, the learned and the ignorant were equally unmindful of the marvel before their very eyes; as unmindful as are the vast majority of men and women at the present day.

All who have made a study of the question are unanimous in the opinion that optical instruments, other than mirrors, were unknown to the ancient world; and that lenses were wholly unknown.

In the first and second centuries of the Christian era there was an abundance of knowledge of mathematics and of optics to make possible the invention of the simple microscope and of appreciating it as such. In works of literature there are hints that men were on the track. For example, Seneca, in his Questiones Naturales (L. I, q. 6), says that "Letters however small and dim are comparatively large and distinct when seen through a glass globe filled with water," and that apples in a vase of water are far more beautiful. He is trying to account for the size of the rainbow and sums it all up by saying that "anything, in fact, that is seen through moisture appears far larger than in reality it is." To Seneca the magnification was the effect of the water and not the effect of the refraction at curved surfaces.

Ancient theories about the eyes. — The microscope and all other optic instruments are intimately bound up with the eyes of the observer, and the brain behind the eyes which gives the final judgment concerning the appearances. This takes us a long journey back into the past for the first understanding of the means by which knowledge of the external world comes to our consciousness.

It was 2500 years ago in the age when Æschylus (525–456), Sophocles (495–406) and Euripides (480–406) wrote their immortal poetry; Phidias wrought forms of beauty out of marble; and Socrates, Plato and Aristotle spoke words of wisdom, that Hippocrates (460–360), the greatest of all ancient physicians, asserted that the so-called "sacred disease," epilepsy, was no more sacred than any other disease; and then he added the brain is the organ by which we think, taste and smell, hear and *see;* through which are joy and sorrow, laughter and tears and, when it is diseased, it brings terror and despair and all insanities.

Nothing in physiology to-day is on firmer ground than that the brain is the final seat of consciousness; and without its healthy action no good vision is possible. Of course, it has always been known that the eyes and light are necessary for vision, but at this time there was much discussion as to the precise means by which objects in the external world could gain their contact with the brain through the eyes. Empedocles thought there must be rays of visual spirits extending from the eyes out to the object and feeling of it, so to speak; Aristotle asserted that the rays of light from the object to the eyes were sufficient; but Plato, to be absolutely safe, assumed that there were needed both the visual rays and the rays of light to make the vision complete.

Six hundred years later, Galen, next in importance to Hippocrates among the ancient physicians, agreed with Empedocles that vision was by means of the visual rays or spirits from the brain and eyes to the object, and gave the cogent argument that all men could appreciate then as now, namely, that objects far off, small or in dim light required much effort to see well as though it were hard work to squeeze out enough visual spirits to make them fully visible.

It was Galen also who argued that the chiasma in the optic nerves from the eyes to the brain was for two great purposes: First, so that if one eye were lost by accident, all the visual spirits could be sent to the remaining eye; and second, it was to answer the puzzling question why with two good eyes two images of everything were not seen. The chiasma, said Galen, is so that the visual rays from the object through the eyes to the brain can be mingled and united so that the brain will have but one image and not two. Furthermore, it is so that the axes of the visual cones will cross and be in one plane, for if these axes are not in one plane there will be seen two images and not one. He gave the simple device of proving this by displacing one axis by pressing on one of the eyeballs with a finger. He asserted also that while normally only one image of an object was seen with the two eyes, this image included more than the image of either eye alone, and gave the experiment of looking at a column first with one eye and then the other, and then both eyes.

Galen gave an excellent anatomical description of the eye and its parts. We still use most of the names he applied, and appreciate what he said about the retina's similarity to brain tissue. He described the vitreus and its hollow cup above to receive the crystalline body. Even before Galen, this crystalline lens was called a lentil-like body, and Galen knew that the curvature was not the same on the two sides. Galen called the eye a most divine instrument, and expressed unbounded admiration for the perfection of the eye for its purpose. Apparently, some people had been finding fault with the eye and saying that they could make a better one themselves. Galen remarks that if they are so much more skillful than the Creator, he would like to see some of the eyes they could make.

Ancient theories about the physical properties of light. — Turning to the physical side, Galen refers to Euclid as the mathematical authority for the straight course of the rays of light, and says that the visual rays are straight like the light rays. He also appeals to the experience of every one who has seen the rays of the sun streaming out through a rift in the clouds.

During the first part of Galen's life there was working in the field of science another giant intellect, Ptolemæus, whose system of astronomy dominated the world for more than 1500 years.

Ptolemæus wrote a book on optics which, it seems to me, is one of the chief landmarks in the history of the subject. Like Euclid he showed that the angles of incidence and reflection were equal, and that the incident and reflected rays were in the same plane; but what for our purposes is of far greater importance, he showed that when light passed from one transparent medium to another, the incident and refracted rays, while they are in the same plane, do not have equal angles with the normal, but that the angle is always less in the denser medium; and he measured the angles for air to water, air to glass and glass to water, and the reverse, and found that no matter in which direction the light passed, the angle was always less in the denser medium. He explained by this bending of the light why it was that a coin in an empty basin, which could not be seen over the edge, became visible when water was added.

For measuring the angles of incidence and refraction, he used a divided circle, and the tables he prepared fill us with admiration for the closeness with which they agree with the results attainable to-day with the most refined apparatus. He not only discussed refraction in bodies with plane surfaces, but also with concave and convex surfaces, and found the rule to hold whatever the shape. It is true that he did not discover the mathematical expression for refraction — that took 1500 years longer — but he showed the facts and stated them with a clearness never since excelled.

Not only did he apply the knowledge to the explanation of the visibility of the coin in the basin, but he showed that from the refraction of the earth's atmosphere, the heavenly bodies were not where they appeared to be, unless they were directly overhead. He showed, too, that if the eye is in the air and the body in a denser medium it will appear enlarged, but if the eye were in the denser medium and the body in air, it would appear smaller. Why, with all his optical knowledge, Ptolemæus did not find the way to make magnifying glasses with curved surfaces, is hard to understand. It took over a thousand years more of effort for that to be accomplished.

In passing, while every one must have the deepest appreciation for the service to the world that the Arabians gave in preserving the science of the Greeks, their additions to scientific knowledge seem very small. For example, in our subject of optics and vision, their statements are almost wholly based on the geometry and optics of Euclid, the optics of Ptolemæus and the structure and functions of the eye as stated by Galen. Their greatest exponent in optics, Alhazen, went back to Aristotle in declaring that vision is by light rays from the object to the eye. He also applied the optics of Ptolemæus to the eye and saw that there must be refraction at the curved surface of the cornea as the light entered the eye.

While the principles of optics and the devising of optical instruments besides mirrors did not command much attention during 1100 years following Ptolemæus and Galen, still some progress had been made by some one, but by whom no one knows.

Theories and experiments of Roger Bacon. — Roger Bacon advocated with the deepest earnestness that the only sure guide to truth was *experiment.* Every theory must stand that acid test before it is wise finally to accept it.

That great 13th century, as it has been called, was one of intense intellectual activity, and was as full of wild guesses and vague dreams as any period in the history of the world, including our own. Roger Bacon tried to put the scientific guesses to the test of experiment as far as he could.

I know that many of us will hear with the sympathy of personal experience what he says in speaking of these experiments. For that time, he was comparatively well off, and he asserts that most of his fortune had been spent for copyists to get the needed books; for calculators to prepare the desired astronomical tables; and, last but not least, to buy the apparatus with which to try the experiments.

The most extravagant claims have been made for Bacon. One would think to read the claims that he was the originator of all scientific knowledge, and the inventor of every piece of scientific apparatus devised before or during his time. He made no such claims. What he believed with all his strength was that the progress of civilization is bound up with a knowledge of science, already at hand or to be gained, and he was filled with zeal to make the knowledge available so that progress might begin at once and proceed with ever increasing speed.

In his enthusiasm he mentioned some things which he thought might be found out, such as flying machines, ships without sails, combinations of lenses to see what was too small or too far off to see with the naked eye, engines of power by the use of explosives. He has, as you know, been credited with the invention of gunpowder, cannon, etc. Here is what he himself says in that connection: "Then wonders can be done by explosive substances. There is one used for amusement in various parts of the world made of powdered saltpeter, sulphur and the charcoal of hazel-wood. For when a roll of parchment about the size of a finger is filled with this powder, it produces a startling noise and flash. If a large instrument were used, the noise and flash would be unbearable, and if the instrument were made of solid material, the violence would be much greater."

To deal specifically with optics, Roger Bacon expounded with great clearness the laws of refraction given by Ptolemæus, and the structure of the eye as given by Galen, and more, he applied the knowledge of refraction to the curved surfaces and structures of the eye in explaining vision. He stated that all the rays reaching the curved surfaces of the cornea and the crystalline lens, except the axial ray of the visual cone, must be bent toward the axis on entering the eye. But this seemed to bring on a trouble which he tried to avoid. The trouble was that if the rays crossed, there would be an inversion, so that what was right would be left, and what left right, and what up would be down, and what down would be up. Here then was a second puzzle to add to that of the single vision with two eyes.

Roger Bacon showed as much skill in getting out of a seemingly tight place as the scientific men of the present day. He assumed that the vitreus with its outer concave surface to receive the crystalline lens was designed on purpose to keep the rays from crossing, and thus to prevent the inversion of the image. It is not so, but it satisfied not only Roger Bacon, but such brilliant minds as Leonardo da Vinci and Maurolycus, and a host of others during the next 400 years.

We may ask what was the fundamental step in optics that Bacon showed. In his own words it was this: "If a man looks at letters and other minute things through crystal glass or other transparent substance in the form of the small part of a sphere . . . he will see the letters far better, and they will appear larger to him, for the angle under which they are seen is greater, and the image is consequently greater. Such an instrument is, therefore, useful for old men and those with weak eyes, for they can see the letters, however small, with sufficient magnitude." . . . Here then is the simple microscope and convex spectacles. For the unnumbered centuries in which the human race had been upon the earth, there never had been any help for giving the sight of youth to the aged and experienced, and the wisest years of life had to be spent in looking at distant things; the near and the minute were only a blur.

So far as I have been able to find, this statement of Roger Bacon concerning the action of artificial lenses for an aid to vision is the first in scientific literature. He does not call these segments of spheres lenses, although he uses the adjective lenticular in describing their form as had been done for the crystalline lens of the eye for over a thousand years.

In leaving the contributions of Roger Bacon to optics, there are two remarkable statements by him of the profoundest significance.

(1) He says light is not composed of material particles, but is a kind of motion, and is not instantaneous in its propagation, but

requires time, although the time is very short. It is transmitted more rapidly in a rarer than in a denser medium on account of the resistance of the density.

(2) He described and gives a diagram showing the passage of the rays of the sun through a flask filled with water, such as had long been used by the physicians for cauterizing; and he says that if any inflammable substance is put at the point where all the rays come together beyond the flask, they will be set on fire. Later he makes this significant statement: "In the fifth place we have to speak of light's action in all its degrees. Its propagation is unequivocal when as light it produces light; but there is equivocal action when it makes something of a different essence, as when light produces heat."

Development of Optical Instruments in Two Groups. — At the time of Roger Bacon's Opus Majus, not only were the principles of reflection and refraction well understood, for plane and curved surfaces, but lenses were actually in hand and it seems as if the way was fairly open for the production of optical instruments. Progress has been from that time on in two closely parallel roads. Sometimes progress has been rapid on one road, and sometimes on the other, depending upon human need.

The two roads serve for two groups of instruments:

The first group contains instruments in which the eye of the observer forms an integral part of the optical train, as with spectacles, the simple and the compound microscope, and the telescope. The second group includes the optical instruments which form real images entirely independent of the eye, like the magic lantern, the projection microscope, the moving picture machine and the photographic camera.

As the most pressing human need was for aid to defective vision, the first development was with spectacles. It is astonishing how soon spectacles came into use after the publication of Roger Bacon's Perspectiva or Optics. This was widely copied and found in many libraries, so that the knowledge soon became available. Even as early as 1299, only about 32 years after Bacon put out his work, there appeared in a manuscript this remarkable passage:

"I am so affected by years that I cannot read or write without those glasses they call spectacles, lately found out for the benefit of poor old men when their eyesight gets weak."

It is also quite modern that cheaper means of producing spectacles were sought. In 1300 the superintendent of arts in Venice found it necessary to forbid the use of glass for making "reading stones" and eye-glasses, for it was believed at that time that only those made of beryl or rock crystal were really effective and not harmful. But in 1301 permission was given to use glass provided the spectacles and reading glasses were sold as glass, not as crystal or beryl.

Naturally, in the beginning the needs of mature persons were especially considered. Their chief difficulty was their growing lack of accommodation that comes with advancing years, and to overcome this, convex spectacles were constructed. Concave spectacles came in later. Two early references to them have been found, the first in the works of Cardinal de Cusa in a chapter called "Beryllus oculare specillum," which reads: "The beryl is a resplendent, colorless and transparent stone to which is given a convex or a *concave* form, and those that look through it succeed in discovering things at first invisible." De Cusa died in 1464, therefore this reference is of a date prior to that.

The second reference to concave spectacles is in the work of Barbaro (1568), p. 192, in which the statement is very specific, for he says, in connection with the construction of a camera for drawing by projection: "Take an old man's glass, convex on both sides, not *concave* like the glasses of youths of short sight."

The next radical step in the development of spectacles was taken by two English astronomers. The first was Thomas Young, a man of many accomplishments, honored equally by the archæologists, physicists, astronomers and physiologists.

In 1800, Young, in experimenting with distances at which lines were sharp to him, found that, when held vertically, the lines were sharp at a distance of twenty-five centimeters, but when horizontal, they had to be held at a distance of only eighteen centimeters. He knew that this meant that some of the refracting surfaces in his eyes had unequal curvatures for the vertical and the horizontal

ZACHARIAS JANSEN 300

Inventor of the Dutch Compound Microscope with convex objective and concave ocular (1590). It gave erect images (fig. 309). Portrait from Petrus Borellus, De Vero Telescopii Inventore, 1655. See also Harting, Mayall, Petri and Carpenter-Dallinger.

JOHANNES KEPLER 301

Astronomer and Optician. Inventor of the compound microscope with convex objective and convex ocular. It gave inverted images (fig. 310), 1611. Portrait from Kepler's Opera Omnia. See Joannis Kepleri, Dioptrice, 1611. "Problema LXXXVI, Duobus convexis majora et distincta præstare visibilia, sed eversa." Opera Omnia, p. 549.

GALILEO GALILEI 302

Astronomer and Physicist. Adaptation of the Dutch telescope construction to a compound microscope with convex objective and concave ocular giving erect images (1610). See Carpenter-Dallinger, Jour. Roy. Micr. Soc., 1889, p. 574; Sedgwick and Tyler, Hist. Science. Portrait, Opere, Vol. I. Milano, 1808

CHRISTIAAN HUYGENS 303

Mathematician. Astronomer and Physicist. Inventor of the Huygenian ocular (1681–1687). Portrait from Œuvres comp. t. vii. See Sedgwick and Tyler, Hist. Science; Encyc. Brit.

CHARLES A. SPENCER 304

Pioneer American Optician. Teacher of Tolles and H. R. Spencer. Producer of microscope objectives of high aperture for resolving power. Manufacturer of glass with special optical qualities, and user of *fluorite* in lens combinations for its optical effects (1851). Proc. Amer. Micr. Soc., 1891, p. 248–249. Memoir by H. L. Smith, Proc. Amer. Micr. Soc., 1882, by Wm. C. Krauss, 1901. Portrait from the original negative in the author's possession.

ROBERT B. TOLLES 305

Student of C. A. Spencer. Producer and advocate of homogeneous immersion objectives for an aperture above 180° in air for their superior resolving power, (1874). Portrait from the memoir of Dr. Blackham. Amer. Micr. Soc., 1884, pp. 41-46. See also General Cox, same volume, pp. 5–39, and Dr. Krauss, 1901, pp. 19–30 with portraits. Mayall, p. 95.

FRANCIS H. WENHAM 306

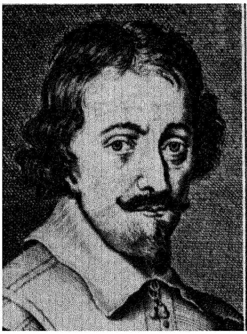
Inventor of the Dark-Field Microscope, 1850–1856, by the use of a paraboloid condenser and central stop to give a hollow cone of light. Advocated necessity of immersion contact of condenser and glass slip for high apertures. Thickness of slip must be equal to the working distance of the condenser. Trans. Micr. Soc. London, III, 1850, pp. 83–90; Quart. Jour. Micr. Soc. 1854, pp. 145–158; 1856, pp. 55–60. Obituary, Jour. Roy. Micr. Soc., 1908, pp. 693–697. Portrait by the courtesy Roy. Micr. Soc. & Ross Ltd.

HERBERT R. SPENCER 307

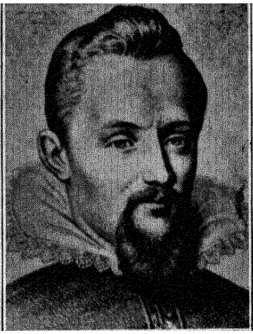
Son and student of Charles A. Spencer. Founder of the Spencer Lens Company. Continued the optical work and traditions of his distinguished father. Portrait from the memoir by Dr. Wm. C. Krauss, Trans. Amer. Micr. Soc., 1901, pp. 19–30. Used fluorite, 1864–1865. Proc. Amer. Micr. Soc. 1891, p. 248.

ERNST ABBE 308

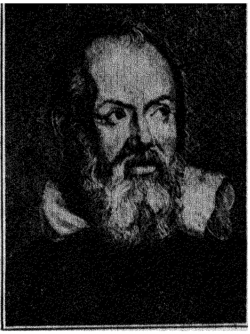
Inventor of the Apochromatic Objectives, and Compensation Oculars for the microscope (1885). Clarifier of discussion and understanding by the use of the expression "Numerical Aperture." Creative genius at the foundation of the Jena Glass Works (1881–1884). Constructive humanitarian in the Zeiss Optical Works. Portrait from Vol. I of the Abhandlungen. See also Jour. Roy. Micr. Soc., 1905, pp. 156–163.



Jansen 300
1590



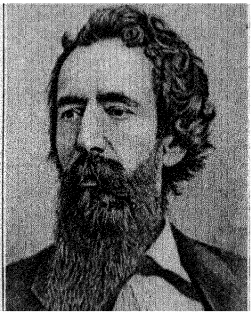
Kepler 301
1571–1630



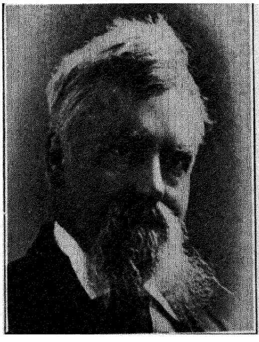
Galileo 302
1564–1642

Huygens 303
1629–1695

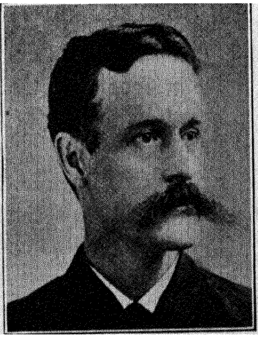
C. A. Spencer 304
1813–1881



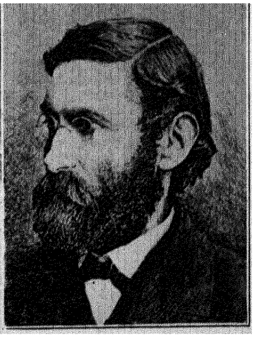
Tolles 305
1822–1883



Wenham 306
1823–1908



H. R. Spencer 307
1849–1900



Abbe 308
1840–1905

axes. He found that this could be compensated by holding a spectacle obliquely before the eye, when the lines would be sharp at the same distance whether they were held vertically or horizontally.

Twenty-five years later, apparently without knowing of Young's experience, George B. Airy found the same difficulty with one of his eyes. The other was short sighted, but otherwise normal. Airy understood the condition as had Young and found obliquity of a spectacle a corrective. He reasoned also that if his eye had a cylindrical instead of a perfect spherical curve, and if a cylindrical spectacle which just balanced the cylindrical curve in the eye were used, there ought to result good vision, and so it proved and has proved for every one corrected for astigmatism since that time.

Up to the end of the 16th century the manufacture of lenses of all shapes was in the hands of the spectacle makers, and from the nature of the work the artisans were men of good intelligence. Naturally, many experiments were tried, and at last in 1590, Jansen, one of the opticians of Middleburg, Holland, got a combination with convex objective and a concave ocular which realized the dream of Roger Bacon, inasmuch as it made small things appear large, and distant things near. As the same instrument served both as a microscope and as a telescope, a little later it was called a microscope-telescope. The possibility of seeing distant objects clearly seemed of immense military importance, so naturally the telescope side was first intensively developed.

Simple microscope. — Every convex lens is or may be used as a microscope, as it aids the eye in seeing an object under an increased visual angle, and hence makes it appear larger than it would if viewed by the naked eye. Hence, when considering the history of the simple microscope, it is evident that that history is the same as the history of convex lenses. The date of the invention is some time before the date of the Opus Majus of Roger Bacon. He speaks of them, not as a wholly new invention of his own time, but as one of the means by which wonderful things can be done. His whole purpose in the discussion was to induce the church to make the fullest use of all the products of science to give the superiority which he felt was the right and the privilege of the Christian world to possess in its efforts for advancing civilization.

The simple lens or the combination of lenses making up a simple microscope may be held in the hand, but ordinarily there is some metal binding and support for the protection of the lens or lenses, and their easier handling or focusing. The common reading glass with its convenient handle (fig. 4) and the tripod (fig. 232) and focusing lens holder (fig. 233) are good examples.

In reading the older literature one often meets with the expression "single microscope." This means a simple microscope, composed of one lens (fig. 145), and is in contrast with the "double microscope," or compound microscope of two lenses or two combinations (objective and ocular, fig. 146).

Dutch and Keplerian compound microscopes. — Each has a convex lens for objective. For ocular the Dutch form has a concave and the Keplerian form a convex lens. The ocular for the Keplerian form is properly a magnifier of the real image, while the concave-lens ocular of the Dutch microscope acts as an amplifier for the objective.

The virtual image is erect with the Dutch, but inverted with the Keplerian microscope.

The Dutch compound microscope. — So far as known at present the first compound microscope invented was composed of two lenses, a convex lens for the objective and a concave lens for the ocular (fig. 309). The convex lens is placed in a position to give a real image of the object, that is, the object is outside the principal focus of the objective, but before the real image is formed, a concave lens (the ocular) is placed in the path of the beam. This makes the rays less convergent and therefore acts as an amplifier, and serves to increase the size of the real image which would be formed by the objective alone. The eye is placed close to the ocular and focuses the real image on the retina. This retinal image is inverted and, therefore, when projected out into space, it seems erect as with the simple microscope.

Very early the two lenses were put into tubes and made capable of being brought together or separated, depending upon the distance of the object to be examined. The nearer the object, the farther apart must be the ocular and objective. There still remains in the ordinary opera glass the original Dutch telescope. If one has an opera glass it is easily demonstrated that it can be used as a microscope by unscrewing the ocular so that it may be separated a considerable distance from the objective. If now the objective is held within 10 to 20 centimeters of an object and the ocular moved back and forth along the axis, the place will be soon found where the image is distinct and it will be seen much enlarged.

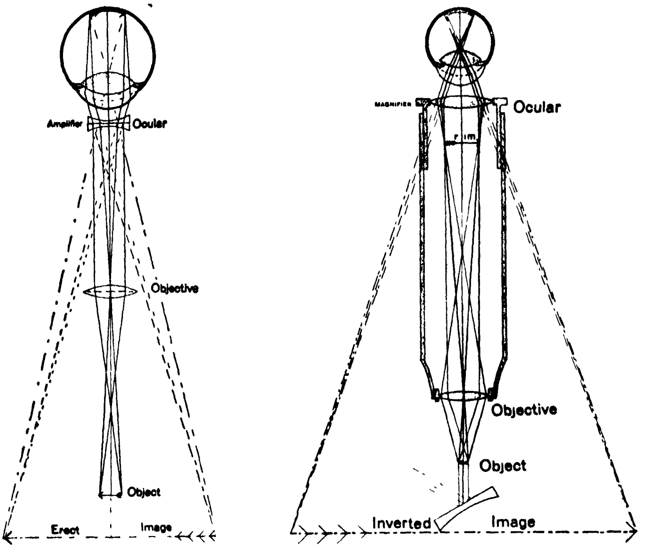


FIG. 309–310. DUTCH AND KEPLERIAN COMPOUND MICROSCOPES FOR COMPARISON.

Each has a convex lens for objective. For ocular the Dutch form has a concave and the Keplerian form a convex lens. The ocular for the Keplerian form is properly a magnifier of the real image, while the concave-lens ocular of the Dutch microscope acts as an amplifier for the objective.

The virtual image is erect with the Dutch, but inverted with the Keplerian microscope.

The name telescope was given sometime before 1618, and the designation microscope in 1625. As every one who used the instrument found that it could be used as a microscope or as a telescope it soon came to be called a telescope-microscope, or a microscope-telescope.

The Keplerian compound microscope. — When the Dutch telescope came to the attention of the astronomer and optician, Kepler, he very quickly saw that the same effect could be brought about by using a convex ocular as well as a convex objective, but that the image would be inverted, the objective serving to produce an enlarged real image and the ocular to magnify that image (fig. 310).

The demonstration of the principles on which such a microscope or telescope could be constructed is to be found in the Dioptrica of Kepler, Proposition LXXXVI. The proposition is: With two convex lenses to show objects larger and inverted.

In Prop. LXXXIX, it is stated that with three convex lenses can be shown objects enlarged and erect. This is the principle of the terrestrial or erecting telescope.

Kepler first showed the real action of the eye as an optical instrument, and that the retinal image must be inverted, and that unless inverted, objects would appear wrong side up. Now we know that is true, for it is an easy demonstration to show, as did Scheiner in 1619–1625, that the retinal image is actually inverted in the eye of an animal or man.

As Kepler showed the actual dioptrics of the eye, he was the first to explain the action of spectacles in correcting the defects of long sight and short sight, viz., to aid the refracting surfaces of the eye to make a sharp image of the object upon the retina.

While Kepler gave the optical demonstration for a microscope or telescope with convex lenses, he, so far as known, did not actually construct such a microscope or telescope. Christopher Scheiner, while he lacked the original genius of Kepler for discovering and expounding principles, had greater mechanical ability. He actually constructed the Keplerian telescope and microscope and used them both for observation and for projecting real images. On page 130 of the Rosa Ursinae (1626–1630) occurs this remarkable passage: "In the same way (i.e., by two convex lenses) was produced that wonderful microscope by which a fly was made as large as an elephant and a flea to the size of a camel."

Binocular Microscopes

From the invention of the telescope-microscope there was dissatisfaction that it was for but one eye, and before 1610 there were made those for both eyes by putting two equal instruments side by side the right distance apart for the eyes of the observer. That arrangement of the Dutch telescope still holds in opera glasses.

One of the first examples shown in pictured form is that of the Cherubin d'Orleans in 1677 (fig. 311). This, as seen from the picture, is a binocular Keplerian microscope, or rather two of them, as both objectives and oculars are of convex lenses. The objectives needing to be close together makes a divergence of the tubes necessary to get the right pupillary distance for the oculars. In general, this form of binocular has been recently revived for dissection, only in the modern form achromatic objectives are used and Huygenian oculars, and by means of prisms the image is made erect.

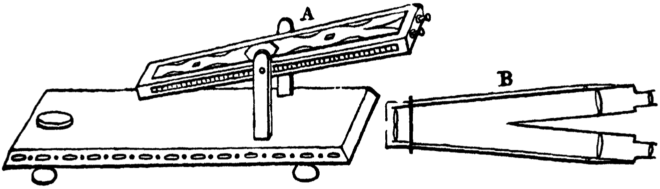


Fig. 311. Binocular Microscope of Cherubin d'Orleans.

A The binocular in its mounting.
B Sectional view showing the two objectives and two oculars.

Only rather large objects can be studied with such binoculars, and the effort to divide the light from a single objective reached success only as late as 1851, when it was worked out by J. L. Riddell of New Orleans. His description and a figure were published in the Quarterly Journal of Microscopical Science in 1854. From that time on successful binocular microscopes have been made. The one of Wenham (fig. 28) in England (1860) enjoyed the greatest favor.

Tolles in 1864–1865 produced his binocular eyepiece, and Nachet in France and Zeiss in Germany produced binocular instruments, but there were defects inherent in the construction of all forms, especially the defect that they could not be used very satisfactorily with high powers, and they were expensive. Finally, in 1902, Mr. F. E. Ives figured and described a form of binocular suitable for all powers, including the highest oil immersion objectives (§ 49). Several recent models have been produced in which the principles he enunciated so clearly have been incorporated (figs. 30–35).

In the first binoculars of the Dutch form, the tubes were parallel, as with opera glasses, but in many of the later forms the tubes have been put at an angle (fig. 33).

MICROSCOPES FOR TWO OR MORE OBSERVERS

The projection microscope with its real images on a screen has been commended from the first invention of projection apparatus because many can see the image at the same time, and the teacher or exhibitor can be sure that the observers are seeing the special things he wishes to show. But in looking into the microscope in the ordinary way only one person can look at a time, even with the ordinary binocular. Therefore there arose the effort to divide the light from the object so that two or more could see the same image at the same time. The use of prisms for dividing the light in the binocular gave the hint, and in 1853 Nachet constructed a microscope for two observers, and another for three observers (see figures of these in Harting and in Robin's work on the microscope, also in the original paper). Harting, 1858, also produced a microscope for two observers. For this the tubes were parallel. By putting them closer together they served for a binocular for one person.

Finally, in his enthusiasm for demonstration, he constructed a microscope in which the beam was divided among four diverging tubes so that four persons could see the same specimen at once.

Within recent years the demand for a way by which two observers could look at once has given rise to two very practical double oculars which are far enough apart so that two can look into the oculars conveniently. One was devised (1910) by Dr. Edinger of Frankfurt and produced by Ernst Leitz in Germany, and the other in 1916, by the Spencer Lens Company of Buffalo, New York. In both these double oculars there is an adjustable pointer so that the exact structure which is to be studied can be indicated; then both teacher and student can be sure that they are talking about the same thing.

OCULARS OR EYE PIECES FOR THE MICROSCOPE

As shown above, the first oculars were of single lenses, — for the Dutch telescope-microscope a concave lens, and for the Keplerian microscope a convex lens (figs. 309–310).

For the Keplerian microscope, which soon became the only one used for microscopic work, all sorts of experiments were tried both for oculars and for objectives. Finally, about 1660, Huygens, the great Dutch astronomer and physicist, designed for the telescope the ocular (figs. 24–25) which now bears his name. It was soon adopted for the microscope and is to this day the most used of any.

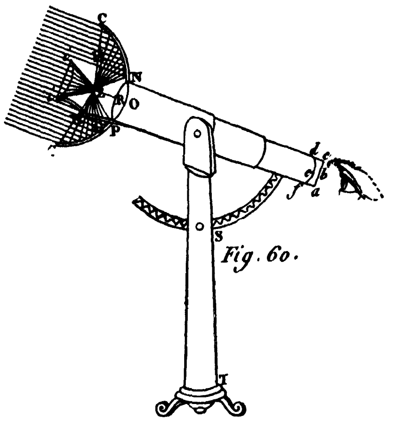


FIG. 312. DESCARTES' DUTCH COMPOUND MICROSCOPE WITH A PARABOLIC MIRROR AND A CONDENSING LENS.

abc, *def* Concave ocular (amplifier).

S T Stand and circle holding the microscope and pointing it toward the sun or other light source.

N O P Convex objective.

C C Parabolic mirror for illuminating opaque objects.

i i Condenser for illuminating transparent objects.

The Ramsden ocular was devised by J. Ramsden (1782) for the telescope and, like the Huygenian, was adapted to the microscope. It has been used especially for the ocular micrometer (figs. 22, 160).

The compensation oculars were invented by Abbe (1885–1886) to go with the apochromatic objectives and to correct the residual defects in the objectives (figs. 23, 114–115).

Mirrors and condensers. — The first objects looked at through the microscope, whether simple or compound, were opaque and were illuminated by light falling upon their surface. For this were used condensing lenses, and plane and concave mirrors. The origin of the mirror is prehistoric. The first were of polished metal and of dark minerals. Those with a metal backing have been known only since about the 12th or 13th century, and those with silver only since about 100 years ago. It is not to be forgotten that still water and other smooth objects in nature serve as mirrors, and have always existed.

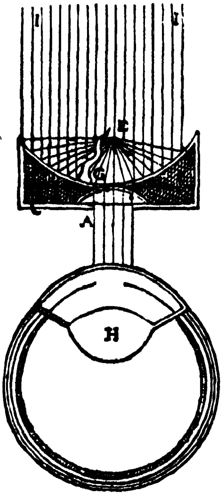


FIG. 313. DESCARTES' SIMPLE MICROSCOPE.

I I Rays of light passing to the reflector.

C The parabolic reflector for illuminating the opaque object.

A The plano-convex lens serving as a magnifier.

G E Pin for holding the opaque object.

H Crystalline lens of the eye.

In Descartes' picture of the Dutch compound microscope (fig. 312) there is a parabolic mirror for lighting the object if opaque, and a condensing lens for transparent objects. Descartes also gives a picture of a simple microscope with a similar concave mirror for illuminating the opaque object (fig. 313). In 1668 Hooke speaks of looking-glasses for illuminating transparent objects for projection. The first pictures of compound microscopes with the mirror, as at present under the stage, are by Hertzel (1712) and Marshall (1718).

A condenser of a single lens or of a combination of lenses for transparent objects dates from the earliest use of the compound microscope, as shown by Descartes' figure. Its importance for adequate lighting has never been lost sight of, as indicated by Brewster (§ 128) and by Nelson (see in collateral reading); and never so thoroughly appreciated as at the present day. The form most common on microscopes is the uncorrected one of Abbe which was first described in the Archiv für Mikr. Anat., Vol. 9, 1873, p. 469.

Achromatism. As pointed out in §§ 257–258, white light, being composed of different wave lengths (figs. 111, 121), must be differently refracted when passed through a prism or lens. To the normal human eye the different waves when separated or dispersed out into groups appear of different colors. Although the nomenclature used by Newton was somewhat different from that now used, he supposed that the refraction of the different waves was in exact accordance with their wave lengths, as is the case with a diffraction grating, and hence there could be no achromatization of dioptric instruments, for when the dispersion was overcome the refraction must also be eliminated. The mistaken belief that the human eye was achromatic, however, kept alive the hope of producing achromatic microscopes and telescopes. Experiments on a large number of transparent substances showed that while all dispersed the light, the dispersion was not the same in all, some affecting one group out of proportion to another. This irregularity gave the clue to the way to accomplish achromatism, for if two or more transparent bodies could be combined to neutralize their dispersive effect without overcoming the mean refraction, it would be possible to make achromatic combinations. This is shown by the course of the beam of white light traversing the two prisms (fig. 112). The first to accomplish the feat in a way to make achromatic telescopes possible was John Dollond (1757). Naturally, the telescope took the lead in the improvement, as it at that time was by far the most important optical instrument. Furthermore, the lenses were relatively large; for in the differentiation of the telescope and microscope the objective of the telescope became progressively larger and that for the microscope progressively smaller. The smaller the lenses the more perfect must be the grinding and polishing, for slight imperfections in their small area introduce obscurations which in the larger surface of the telescope lenses would be negligible (§ 272, fig. 119). But the microscope makers undertook the task in several different countries, — England, France, Russia, Holland, Germany and Italy — and from 1759 to 1824 were tireless in their efforts. Finally Selligue laid before the French Academy the result of his efforts with the help of the practical opticians, Vincent and Charles Chevalier. From that time on, achromatic objectives became more and more common for microscopes, although from their small aperture they were not liked by some workers so well as the more brilliant, uncorrected lenses.

In our own country, Charles A. Spencer took the lead in trying to overcome the lack of brilliancy in achromatic objectives. He, too, early realized and grasped the importance of aperture for the microscopic objective. He realized also that for the balancing of the dispersions and refractions to make true achromatic combinations, it was necessary to have materials for lenses with special properties. He worked in two directions. One was the use of the natural mineral fluorite whose properties had been pointed out by Brewster (§ 259a) and the other was the production of new forms of glass with specially desired optical qualities.

It fills one with admiration to think of this genius with small means working alone in his cramped quarters trying to make new forms of glass, which with the old forms and with natural minerals would enable him to produce the objectives of his dream with large aperture and perfect color and spherical correction. While his success, and that of his pupil Tolles, were certainly great in producing the highest type of objective for the telescope and microscope with the materials already to be had, his glass making did not bring him all that he wanted. It was reserved for the optical works of Zeiss and the genius of Abbe, with the help of the practical glass maker Schott and the liberality of the German government, finally to overcome the difficulties in making new forms of glass with specially desired qualities of dispersion and refraction; and even then it was necessary to go back to the natural mineral fluorite to make possible the apochromatic objectives. Those interested are recommended to read the work of Hovestadt on the new Jena glass.

Immersion objectives. In the development of any art the science needed almost always lags behind, and is developed in most cases to explain what has already been discovered by the hard and roundabout method of "trial and error." This was the case with immersion objectives. Amici in Italy and David Brewster in Great Britain were busy in trying to improve microscope objectives by any feasible method. They used all sorts of liquids for immersion. Water was one of the most successful and still holds its own.

The advantage of the immersion principle gradually became understood to be the possibility of increasing the aperture under which the object could be viewed. The final step by which the aperture could be pushed to the limit of human skill in figuring the lenses came when Mr. Tolles (1871–1874) showed in the clearest manner the possibility of making such objectives and increasing the aperture by means of homogeneous contact between the condenser and the slide or object and between the object or cover-glass and the front lens of the objective. The matter is well stated by Hon. J. D. Cox in his presidential address before the American Microscopical Society for 1884 (pp. 5–39), and in Mr. Mayall's Cantor Lectures on the History of the Microscope (1885). On p. 96 Mayall says: "If priority of publication of the formula on which homogeneous immersion objectives could be produced carries with it the title of inventor, then Mr. R. B. Tolles stands alone as inventor; but he not only published the formula, he constructed objectives on it." The formula was submitted with the objective in 1874. The homogeneous immersion objectives of Zeiss came out in 1878.

Many substances have been tried for the homogeneous fluid. Thickened cedar-wood oil has proved most satisfactory. Mr. Tolles used Canada balsam; if one is out of cedar-wood oil and has Canada balsam of moderate thickness, good results can be obtained by using the balsam as an immersion liquid with ordinary light. As shown above (§ 309), none of the regular homogeneous immersion liquids will answer for the immersion medium with the ultra-violet microscope. Petrolatum has nearly the right refractive index, and is non-fluorescing, therefore it answers well for the immersing fluid in ultra-violet work. It is also used by many for the usual routine examinations with the oil immersion objectives, but one cannot get the most perfect images when it is used (§ 269).

THE DARK-FIELD MICROSCOPE

In the earliest literature giving directions for the use of optical instruments, there is made over and over again the statement that for the clearest images no light should reach the eye except from the object itself. But when the object is on a white background, or when lighted by rays from behind and on all sides, filling the whole field of view, it is evident that the light from the object is only a small part of that which enters the eye, and the fine details are wholly obliterated or only dimly seen. To overcome this difficulty two means have been employed: — First, myriads of dyes have been invented to stain the delicate parts of the microscopic objects so that color images are given in the bright field. The second method is an application to microscopy of the knowledge gained in astronomy — that is, to view the objects only by the light which they themselves send into the microscope. Of course, if the objects are truly self-luminous, as are the fixed stars in astronomy, no accessory light is needed, but if, as with the planets in the sky, objects without any intrinsic light, must in some way be illuminated brightly by an outside source, and in that way objects become visible as if self-luminous, by the extrinsic light which they reflect, refract or diffract into the microscope, just as the planets deflect the light from the sun to the earth.

With the sky at night, the back ground will be dark and appear like empty space, and the whole attention can be given to the shining objects. If the objects are too small to be resolved by the microscope used, then they will appear simply as points of light, as with the ultra-microscope, but if they come within the resolving power of the microscope, all the finest details will be brought out with striking clearness. Naturally, to get this dark-field illumination the light from the source must be of so great obliquity that none of it can enter the microscope objective directly, and it must be of sufficient brilliancy so that the objects to be studied will be bright enough to be clearly visible. This dark-field microscopy was begun by Lister in 1830, and by Reade in 1837, and made available for the highest powers by Wenham in 1850–1856.

It seems to me after many years of experience with all the dyes used in microscopy for bright-field work, and with all the dark-field methods so far devised, that the future physicist, chemist and biologist will feel as much handicapped without the ultra-microscope and the dark-field microscope as would the astronomers if they had no clear, dark nights, and could work only in the daytime.

Oculars for Use with Spectacles

Wherever in the brain the final visual effects may be interpreted, it has been recognized since the time of Kepler that for the clearest vision there must first be formed a perfect image on the retina of the eye, and that the entire optical and accommodating mechanism of the eye exists for the sole purpose of producing a sharp retinal image. Kepler, Young and Airy showed exactly what concave, convex and cylindrical spectacles did to aid in giving a perfect retinal image when there were defects of short sight, long sight or astigmatism. Now, as the optician strives to make his instruments capable of giving, with a normal eye, a perfect retinal image, it follows logically that with an imperfect eye in the optical train no perfect retinal image is possible, no matter how good the optician's work has been. If, then, the observer's eyes must be helped by spectacles to get a perfect retinal image, the spectacles should be worn when looking into an optical instrument as well as when reading or using the eyes for any other accurate vision. Probably every optician would agree to this as a general proposition, but strange to say, until very recently, microscope makers, at least, have constructed their oculars so that it is almost or wholly impossible to use one's spectacles when looking into the microscope. That is, they have constructed them so that the eyepoint or exit-pupil is so near the eyelens that spectacles, especially those of toric form, cannot be worn because they keep the eye too far from the eyelens of the ocular. Furthermore, some of the best makers, when the oculars were constructed with high eyepoints to give the best effects, have added a perforated tube to the top of the ocular so that the spectacled user even than had to remove his spectacles.

One English optical house has listened to the appeals of their toric-spectacled patrons, and has produced a full series of oculars with eyepoints high enough so that toric spectacles can be worn with comfort when using the microscope.

I have appealed to our American opticians to give the spectacle users — and practically every one doing serious research must wear spectacles — this measure of assistance, and I hope the members of the Optical Society will add their influence.

ARTIFICIAL DAYLIGHT

I suppose that every one will agree that the human eye was created or developed for daylight; and what it required untold ages to evolve, naturally resists rapid change. In the short days of fall and winter, and in the dimness of foggy weather in many regions, the daylight is distressingly short or inadequate for the exacting work of the modern world, hence the artificial lights to gain extention of time and efficiency. Even the best artificial lights are so unlike sunlight that the eyes are put to a great strain.

To remedy this trouble many efforts have been made to give daylight qualities to the artificial lights which must be used. Fortunately, within the last few years such artificial daylight has been made available at a very moderate cost. Certainly for users of the microscope it is a great boon, and from much experience it is believed that with this help the eyes of the experts will be able to serve their owners for a longer time to carry on their researches, much to the advantage of the individual and of the community.

REAL IMAGES AND PROJECTION

The production of real images by means of a naked aperture and by means of a lens were the beginnings of the magic lantern, the photographic camera, the *projection microscope* and the drawing camera.

As shown elsewhere (Optic Projection, p. 673), the production of real images in dark places by means of an aperture or hole in the wall is a purely natural phenomenon. The systematic utilization of this phenomenon by man had its beginnings in the sixteenth and seventeenth centuries. The first certain statement of the use of a lens in the aperture to make the picture clear and vivid occurs in the work of Daniel Barbaro on perspective.

From this time on, a lens is always used for projection. At first the images were smaller than the object, as naturally only the brightly lighted objects in the exterior world were projected, but as artificial and natural light were used to illuminate smaller and smaller objects, many of which were transparent, and the projection lenses were made of shorter focus, the images became larger than the object. Finally (1665), when the apparatus became small, and only the object and lens and light were enclosed and the image was on a screen outside, the magnifying action seemed like that of a microscope, and Milliet de Chales, in speaking of the magic lantern of Walgensten, says (Vol. II, p. 667): "In this machine you have a kind of microscope," and Zahn, p. 255, in discussing the magic lantern, says: "It is a kind of microscope." Both authors point out the great advantage this kind of microscope has over the ordinary one in that many persons can see the image at the same time. Kepler (1611) showed that the Dutch telescope-microscope and also his own combination of convex lenses, could be used for projecting images. Scheiner (1626–1630) used them for projecting images of the sun so that he could draw the spots. See also Hooke, Trans. Roy. Soc., 1668, p. 741.

Naturally, with the perfecting of objectives (1824 and onward), and the finding of more powerful artificial lights (lime light, 1824, electric light, especially since 1880), the projection microscope is coming to be used more and more.

The use of real-image forming optical appliances is increasing with ever accelerated velocity in our own time. To realize this one has only to think of the photographic cameras in the hands, not only of experts, but of old and young everywhere, and to think of the moving picture machines in every village and in many private homes. So, too, the magic lantern is a part of the regular outfit of many churches and societies and practically every high school and college in the land; and the projection microscope for showing the minute details of the objects to be studied finds an honorable place in laboratories of all universities.

Recently projection apparatus has found a welcome in testing laboratories, much to the advantage and comfort of those making the tests, for the real images can be seen with both eyes and the head and shoulders held in a natural, unstrained position (§ 444).

Its aid in getting accurate drawings is now more appreciated than ever. It also serves to obtain photographs of microscopic objects which give the minutest details with a delicacy and accuracy that the human artist finds difficult to approach.

The first drawings made by the aid of the microscope were free-hand. Examples of the drawings may be seen in the work of Borellus, and in facsimiles shown in the Journal of the Royal Microscopical Society, 1915, pp. 317–340. The desire for accuracy and ease in tracing outlines of microscopic images comparable with those so easily attained with the real images of the projection microscope led to the invention of the *camera lucida*, by which the microscopic field and the drawing field, pencil, etc., can be superposed. The first one invented is still used. It is the Wollaston form (fig. 168), and was described by Wollaston in Nicholson's Journal, 1807, pp. 1–5. The other form shown in fig. 169 was described in principle by G. Burch, Jour. Quek. Micr. Club, 1878, p. 47; and by Dippel in the Bot. Centrlbl., 1882, pp. 242–3.

Drawing with the projection apparatus has been practised from its first invention. Indeed, in all those who described such apparatus, the great help that was to be gained in drawing was emphasized. Both eyes can be used, and perfect freedom of the artist is enjoyed, which is in marked contrast with camera lucida drawing. For the early appreciation of projection apparatus and the camera obscura for drawing see: Barbaro, 1568; Kepler, 1611; Scheiner, 1626–1630; Robert Hooke, 1668; Henry Baker, 1742; G. Adams, 1746; Goring and Pritchard, 1837; Chevalier, 1839.

Daniel Barbaro. — In his work, La pratica della perspettiva, Venice, 1568, Ch. V, p. 192, Barbaro says: "Take an old man's glass, convex on both sides, not concave like the glasses of youths of short sight, fix the convex glass in a hole, close all the windows so that no light may enter except through the lens. Now take a sheet of white paper and bring it toward the lens until all outside the house is clearly seen. When the proper position is found you will see the images on the paper as they are, and the gradations in colors, shadows, movements, clouds, the rippling of waters, birds flying, and everything that can be seen. For this experiment the sun must be clear and bright, for the sunlight has great power in bringing out the images. You can draw on the paper with a pencil all the perspective, and the shading and coloring according to nature."

Johannes Kepler. — In Reliquiae Wottonianae, edited by Izaak Walton, London, 1672, pp. 298–300. In a letter to his kinsman, Francis Bacon: "I have your Lordship's letters dated the 20th of October (1620). I lay a night at

Lintz. . . . There I found *Keplar*, a Man Famous in the Sciences, as your Lordship knows, to whom I purpose to convey from hence one of your Books, that he may see we have some of our own that can Honor our King, as well as he hath done with his *Harmanica*. In this Mans study I was much taken with the draught of a Landskip on a piece of Paper, methoughts Masterly done: whereof inquiring the Author, he bewrayed with a smile, it was himself; adding, he had done it, *Non tanquam Pictor, sed tanquam Mathematicus*. This set me on Fire: At last he told me how. He hath a little black Tent (of what stuff is not much importing) which he can suddenly set up where he will in a Field, and it is convertible (like a Wind-mill) to all Quarters at Pleasure, capable of not much more than one Man, as I conceive, and perhaps at no great ease; exactly close and dark, save at one hole, about an Inch and a half in the *Diameter*, to which he applies a long perspective Trunk, with a Convex glass fitted to the said hole, and the concave taken out at the other end, which extendeth to about the middle of this erected Tent, through which the visible Radiations of all the Objects without, are intromitted, falling upon a Paper, which is accommodated to receive them, and so he traceth them with his Pen in their natural Appearance, turning his little Tent round by Degrees, till he hath designed the whole Aspect of the Field. This I have described to your Lordship, because I think there might be good use made of it for Chorography: for otherwise, to make Landskips by it were illiberal; though surely no Painter can do them so precisely."

Henry Baker. — The Microscope Made Easy, 1742. On page 25 occurs this "Such too as have no skill in drawing may, by this contrivance [projection microscope], easily sketch out the exact figure of an object they have a mind to preserve a picture of; since they need only fasten a paper upon a screen and trace it out thereon either with a pen or pencil as it appears before them." This old book has an abundance of illustrations and contains a mine of good suggestions.

SPECTROSCOPE, POLARIZING MICROSCOPE, ULTRA-VIOLET MICROSCOPE

Spectroscope for use with a microscope. Since the fundamental studies by Newton on the colors in white light by the aid of a prism in 1666, all kinds of light have been subjected to spectral analysis and many important facts concerning the physical world have been discovered. For the investigation of minute objects, a special form of spectroscope has been devised for use with the microscope. It is of the direct-vision form (§§ 274, fig. 120) and was devised and perfected by Sorby and Huggings (1865) and perfected later by Browning, Swift, Ward and Abbe, etc. Spectroscopes for the microscope have been named: Micro-spectroscopes, spectral oculars, and Mr. Dallinger suggests that the name spectro-microscope be used. This would bring the name in harmony with polarizing microscope, ultra-violet microscope, etc.

Polarizing Microscope. Since 1808–10, when Malus found that light reflected from glass surfaces had peculiar properties which he named polarization, an immense amount of investigation has been undertaken to find out the meaning of polarization, and the phenomena which polarized light produces. Much study and many investigations have been applied to the means of polarizing light, and many phenomena when first discovered have been brought in line with this peculiarity, for example, the double refraction in calcite discovered and described by Bartholinus in 1669. The investigations have been greatly simplified and made exact by the invention of the polarizing and analyzing prisms of Wm. Nicol, described by him in 1828.

Sir David Brewster sought to discover the effects of polarized light upon small objects, and devised a method of doing so with a simple microscope in 1816. It was not, however, until after the Nicol Prism was invented in 1828 that a completely successful application of polarized light was made to the compound microscope. This was accomplished by Henry Fox Talbot in 1834. First he used tourmaline, but the color was objectionable. Then he used Nicol prisms with complete success. One prism was put under the stage to polarize the light and a similar one over the ocular to analyze it. He called the two prisms (figs. 91–92) polarizers. In a paper in 1836 Talbot gave the true explanation of the single beam shown by the Nicol prism, asserting that one of the beams was wholly removed by total internal reflection (§ 216).

The information concerning the physical character of objects revealed by the use of polarized light was early appreciated, and its use advocated with great earnestness by the early workers, Brewster, Talbot, Quekett, etc. Lately there has appeared an entire volume of over 500 pages dealing with animal tissues and organs (W. J. Schmidt). Drs. Chamot and Mason show in their Micro-Chemistry what an indispensable aid the polarizing microscope is in chemistry.

INVISIBLE RADIATION

The discovery that outside the band of visible light were similar radiations which were invisible, was epoch making. The first of these discoveries was by William Herschel in 1801, when he found the infra-red of the solar light, or the invisible heat rays of sunlight. The second, stimulated by Herschel's work, was the discovery by Johann Wilhelm Ritter in 1801 to 1803 of the ultra-violet radiations beyond the violet end of the spectrum. As the infra-red radiations were found by their heating effect, and still are called heat rays, the ultra-violet was discovered from the action on chlorid of silver, and was called actinic or chemical radiation.

These heat rays and chemical rays have, since their discovery, played a great rôle in physics, and are destined to play an equally great rôle in physiology.

Ritter says that he found the chemical action, the 22d of February, 1801, by the use of one of Newton's prisms in a region below the violet (ausserhalb des Violet). (Annalen der Physik, Bd. 7, p. 527 and p. 409.)

It is a long road leading to the present activity in experimenting with ultra-violet radiation, and now the activity is at a highly increased velocity. For the scores of papers dealing with the physiological side, one can find full information in the Quarterly Cumulative Index Medicus.

In 1833, David Brewster found that certain substances glowed with visible light when acted upon by ultra-violet. He called the effect "internal dispersion." In 1852 came the fundamental work of George Gilbert Stokes, "On the Change of Refrangibility of Light," and he proposed to call the change by which objects gave out light visible to the eye when acted on by the invisible radiation, "Fluorescence" and that name is now universally employed.

The ultra-violet or fluorescence microscope utilizing radiation of 250 mμ to 410 mμ wave length can now be used in every laboratory. The capillary mercury arcs (fig. 129 A) are as simple to use as any arc or mazda light. For elimination of visible light from the mercury light there must be an ultra-violet filter like the red-purple corex A (fig. 129 B). For photo-micrography there are other filters to transmit only special mercury spectral lines. The condenser must be of quartz and for most purposes must have a dark-field element at the top as illustrated in figures 126, 280. The slips on which the objects are mounted must be of quartz or of corex or other ultra-violet transmitting glass.

The cover-glasses, objectives and oculars ordinarily used with a microscope are employed. (fig. 125, 128).

The objects to be studied are either fluorescent and thus send their visible light into the microscope, or, if non-fluorescent, they are observed by ordinary light to see if any changes have taken place due to the ultra-violet irradiation.

Ultra-Violet for Photography. It was thoroughly appreciated by the leading microscopists of the 19th century that the resolution of the microscope was bound up with the wave length of the light used. That being assumed, it was felt certain that if one could make use of the short ultra-violet radiation, finer and finer details could be brought out. As these radiations are invisible to the eye, recourse was had to photography, for the salts of silver were sensitive to these shorter waves. Hence arose ultra-violet photography. But it is only for the longer ultra-violet waves that glass is transparent. For still shorter waves it is necessary to use quartz. Then a source of radiation had to be found which produces the maximum number of ultra-violet waves. The mercury arc is good for many purposes where the longer waves are desired. For still shorter ones the arc light with cadmium electrodes gives abundant waves down as short as 0.275μ.

To return to the microscope, in 1903–1904 Köhler described and the Zeiss Works produced a microscope with quartz lenses by which photo-micrographs could be made with ultra-violet. In America Drs. Ernst and Wolbach published an account of their work and results in this ultra-violet photo-micrography, and quite recently Dr. Francis F. Lucas has described an apparatus for photo-micrography and has produced many wonderful photographs of living cells using the ultra-violet.

As a final word in the history and future promise of the services the microscope has given and it is believed will give, there are two reasons for astonishment. The first is that mankind was so late in discovering the laws of refraction, and the possibilities which it might lead to in the production of lenses and optical instruments.

Secondly, it is astonishing to think of the rapid progress that has been made since the possibilities of lenses were discovered some six hundred years ago, and especially during the last three hundred years, since the compound microscope, the telescope and achromatic instruments have been invented.

And finally, with the abundance of stains and the newer methods of physical analysis of the structure and action of living, fresh and fixed material with the spectroscope, the dark-field microscope, the polarizing and the ultra-violet microscope, one can look forward with confidence to still greater discoveries, and with a corresponding deeper insight into the complex structure and the marvelous functions of living things.

(In the accompanying references to the history of optics and the microscope, one will find the sources of information on which this brief history is founded).

COLLATERAL READING FOR CHAPTER XV

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MALUS. — Polarized light, 1808–1810.

NEWTON, SIR ISAAC. — Optics.

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RIDDELL. — First one to produce a practical, single objective, binocular microscope, 1853.

RITTER, J. W. — Discovered ultra-violet, 1801.

SCHEINER. CHR. — The first to make a Keplerian compound microscope. 1619–1626.

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WENHAM. — Inventor of means for dark-field microscopy with all powers. 1850–1856.

YOUNG, THOMAS. — The first to point out astigmatism in the eye, and gave a means of correcting it (1800). See also Airy.

See the brief statements concerning the portraits p. 556. See also collateral reading, pp. 50, 168, 257.

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It is most earnestly recommended that those using the microscope pay particular attention to the booklets and the catalogs put out by the manufacturers of microscopes. They know what they are talking about.

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Raspail has been called the father of micro-chemistry. His work certainly gave the study a tremendous forward push. His discussion of the theory and use of the microscope in tome 1, pp. 174–281, is a masterpiece for the time (1838).

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Interpolation with Natural Sines: — If one cannot find a sine exactly corresponding with an angle in the table, or an angle corresponding with a sine found in solving a problem, the sine or angle can be closely approximated by the method of *Interpolation:* Find the sine in the table nearest the sine whose angle is to be determined. Get the difference of the sines of the angles greater and less than the sine whose angle is to be determined. That will give the increase of sine for that region of the arc for 15 minutes. Divide this increase by 15 and it will give with approximate accuracy the increase for 1 minute. Now get the difference between the sine whose angle is to be determined and the sine just below it in value. Divide this difference by the amount found necessary for an increase in angle of 1 minute and the quotient will give the number of minutes the sine is greater than the next lower sine whose angle is known. Add this number of minutes to the angle of the next lower sine and the sum will represent the desired angle. Or if the sine whose angle is to be found is nearer in size to the sine just greater, proceed exactly as before, getting the difference in the sines, but subtract the number of minutes of difference and the result will give the angle sought. For example, take the case in Section 108 where the sine of the angle of 28° 54′ is given as 0.48327. If one consults the table the nearest sines found are 0.48099, the sine of 28° 45′, and 0.48481, the sine of 29°. Evidently then the angle sought must lie between 28° 45′, and 29°. If the difference between 0.48481 and 0.48099 is obtained, 0.48481 − 0.48099 = 0.00382, and if this increase for 15′ be divided by 15 it will give the increase for 1 minute; 0.00382 ÷ 15 = 0.000254. Now the difference between the sine whose angle is to be found and the next lower sine is 0.48327 −0.48099 = 0.00382. If this difference be divided by the amount found necessary for 1 minute it will give the total minutes above 28° 45′, 0.00228 ÷ 0.000254 = 9. That is, the angle sought is 9 minutes greater than 28° 45′ = 28° 54′.

Table of Metric and English Measures: —

Meter (unit of length) = 100 centimeters; 1000 millimeters; 1,000,000 microns (μ); 39.3700 inches; 3.2808 feet.
Centimeter (cm.) = 10 millimeters; 10,000 microns; 0.01 meter; 0.3937 (2/5) inch.
Millimeter, (mm.) = 1,000 microns (μ); 0.1 cm.; 0.001 meter; 0.03937 (1/25 inch).
Micron (unit of length in micrometry) (μ) (§246) = 0.001, one thousandth of a millimeter; 0.000001, one millionth of a meter; 0.00003937 (1/25000) inch.
Kilometer = 1000 meters; 0.621 or 5/8 mile.

Liter (unit of capacity) = 1000 cubic centimeters (or milliliters); 1 quart approximately.
Gram (unit of weight) = 1 cc. of water; 15.432 grains.
Kilogram = 1000 grams; 2.2046 (2 1/5 lbs.).
Yard, 3 feet, 36 inches; 0.9144 meter; 91.4399 cm.
Foot = 1/3 yard; 12 inches; 0.3048 meter; 30.48 cm.
Inch = 1/36 yard; 1/12 foot; 2.54 cm.; 25.4 mm.
Mile = 1760 yards; 5280 feet; 1.61 kilometers.

Quart = 1/4 gallon; 2 pints; 32 fluid ounces; 0.947 liter (947 cc.). (U. S. liquid).
Fluid ounce = 8 fluidrachms; 1/32 of a quart; 1/16 pint; 29.574 cubic centimeters (30 cc. approximately).
Ounce avoirdupois = 437 1/2 grains; 28.349 grams.
Ounce apothecaries or *Troy* = 480 grains; 31.103 grams.
Pound (avoirdupois) = 16 ounces, 453.6 grams.

To Change from Centigrade to Fahrenheit and the Reverse: —

From centigrade to Fahrenheit: Multiply the degrees centigrade by 9/5 and add 32. Example: 20° C. = 20 × 9/5 + 32 or 68° F.

From Fahrenheit to centigrade: Subtract 32 and multiply by 5/9. Example: 77° F. = 77 − 32 × 5/9 or 25° C.

To change from centigrade to absolute temperature and the reverse: Add 273 to the degrees in centigrade and the sum will be the absolute temperature. Example. Ice melts at 0° C. or 0° + 273° = 273° absolute, and water boils at 100° C. or 100° + 273° = 373° absolute. If the absolute temperature is given subtract 273 and the result will be the temperature on the centigrade scale. Example: Ice melts at 273° absolute, 273° − 273° = 0°, that is, ice melts at 0° C. See Fig 45, where absolute temperature is given.

TABLE OF NATURAL SINES

Compiled from Prof. G. W. Jones' Logarithmic Tables

Minutes		Degrees and Quarter Degrees up to 90°											
1′	0.00029	1°	0.01745	16°,	0.27564	31°,,	0.51504	46°,	0.71934	61°,	0.87462	76°,	0.97030
2	0.00058	1°,15′	0.02181	16°,15′	0.27983	31°,15′	0.51877	46°,15′	0.72236	61°,15′	0.87673	76°,15′	0.97134
3	0.00087	1,30	0.02618	16,30	0.28402	31,30	0.52250	46,30	0.72537	61,30	0.87882	76,30	0.97237
4	0.00116	1,45	0.03054	16,45	0.28820	31,45	0.52621	46,45	0.72837	61,45	0.88089	76,45	0.97338
5	0.00145	2	0.03490	17	0.29237	32	0.52992	47	0.73135	62	0.88295	77	0.97437
6	0.00175	2,15	0.03926	17,15	0.29654	32,15	0.53361	47,15	0.73432	62,15	0.88499	77,15	0.97534
7	0.00204	2,30	0.04362	17,30	0.30071	32,30	0.53730	47,30	0.73728	62,30	0.88701	77,30	0.97630
8	0.00233	2,45	0.04798	17,45	0.30486	32,45	0.54097	47,45	0.74022	62,45	0.88902	77,45	0.97723
9	0.00262	3	0.05234	18	0.30902	33	0.54464	48	0.74314	63	0.89101	78	0.97815
10	0.00291	3,15	0.05669	18,15	0.31316	33,15	0.54829	48,15	0.74606	63,15	0.89298	78,15	0.97905
11	0.00320	3,30	0.06105	18,30	0.31730	33,30	0.55194	48,30	0.74896	63,30	0.89493	78,30	0.97992
12	0.00349	3,45	0.06540	18,45	0.32144	33,45	0.55557	48,45	0.75184	63,45	0.89687	78,45	0.98079
13	0.00378	4	0.06976	19	0.32557	34	0.55919	49	0.75471	64	0.89879	79	0.98163
14	0.00407	4,15	0.07411	19,15	0.32969	34,15	0.56280	49,15	0.75756	64,15	0.90070	79,15	0.98245
15	0.00436	4,30	0.07846	19,30	0.33381	34,30	0.56641	49,30	0.76041	64,30	0.90259	79,30	0.98325
16	0.00465	4,45	0.08281	19,45	0.33792	34,45	0.57000	49,45	0.76323	64,45	0.90446	79,45	0.98404
17	0.00495	5	0.08716	20	0.34202	35	0.57358	50	0.76604	65	0.90631	80	0.98481
18	0.00524	5,15	0.09150	20,15	0.34612	35,15	0.57715	50,15	0.76884	65,15	0.90814	80,15	0.98556
19	0.00553	5,30	0.09585	20,30	0.35021	35,30	0.58070	50,30	0.77162	65,30	0.90996	80,30	0.98629
20	0.00582	5,45	0.10019	20,45	0.35429	35,45	0.58425	50,45	0.77439	65,45	0.91176	80,45	0.98700
21	0.00611	6	0.10453	21	0.35837	36	0.58779	51	0.77715	66	0.91355	81	0.98769
22	0.00640	6,15	0.10887	21,15	0.36244	36,15	0.59131	51,15	0.77988	66,15	0.91531	81,15	0.98836
23	0 00669	6,30	0.11320	21,30	0.36650	36,30	0.59482	51,30	0.78261	66,30	0.91706	81,30	0.98902
24	0.00698	6.45	0.11754	21,45	0.37056	36,45	0.59832	51,45	0.78532	66,45	0.91879	81,45	0.98965
25	0.00727	7	0.12187	22	0.37461	37	0.60182	52	0.78801	67	0.92050	82	0.99027
26	0.00756	7,15	0.12620	22,15	0.37865	37,15	0.60529	52,15	0.79069	67,15	0.92220	82,15	0.99087
27	0.00785	7,30	0.13053	22,30	0.38268	37,30	0.60876	52,30	0.79335	67,30	0.92388	82,30	0.99144
28	0.00814	7,45	0.13485	22,45	0.38671	37,45	0.61222	52,45	0.79600	67,45	0.92554	82,45	0.99200
29	0.00844	8	0.13917	23	0.39073	38	0.61566	53	0.79864	68	0.92718	83	0.99255
30	0.00873	8,15	0.14349	23,15	0.39474	38,15	0.61909	53,15	0.80125	68,15	0.92881	83,15	0.99307
31	0.00902	8,30	0.14781	23,30	0.39875	38,30	0.62251	53,30	0.80386	68,30	0.93042	83,30	0.99357
32	0.00931	8,45	0.15212	23,45	0.40275	38,45	0.62592	53,45	0.80644	68,45	0.93201	83,45	0.99406
33	0.00960	9	0.15643	24	0.40674	39	0.62932	54	0.80902	69	0.93358	84	0.99452
34	0.00989	9,15	0.16074	24,15	0.41072	39,15	0.63271	54,15	0.81157	69,15	0.93514	84,15	0.99497
35	0.01018	9,30	0.16505	24,30	0.41469	39,30	0.63608	54,30	0.81412	69,30	0.93667	84,30	0.99540
36	0.01047	9,45	0.16935	24,45	0.41866	39,45	0.63944	54,45	0.81664	69,45	0.93819	84,45	0.99580
37	0.01076	10	0.17365	25	0.42262	40	0.64279	55	0.81915	70	0.93969	85	0.99619
38	0.01105	10,15	0.17794	25,15	0.42657	40,15	0.64612	55,15	0.82165	70,15	0.94118	85,15	0.99657
39	0.01134	10,30	0.18224	25,30	0.43051	40,30	0.64945	55,30	0.82413	70,30	0.94264	85,30	0.99692
40	0.01164	10,45	0.18652	25,45	0.43445	40,45	0.65276	55,45	0.82659	70,45	0.94409	85,45	0.99725
41	0.01193	11	0.19081	26	0.43837	41	0.65606	56	0.82904	71	0.94552	86	0.99756
42	0.01222	11,15	0.19509	26,15	0.44229	41,15	0.65935	56,15	0,83147	71,15	0.94693	86,15	0.99786
43	0.01251	11,30	0.19937	26,30	0.44620	41,30	0.66262	56,30	0.83389	71,30	0.94832	86,30	0.99813
44	0.01280	11,45	0.20364	26,45	0.45010	41,45	0.66588	56,45	0.83629	71,45	0.94970	86,45	0.99839
45	0.01309	12	0.20791	27	0.45399	42	0.66913	57	0.83867	72	0.95106	87	0.99863
46	0.01338	12,15	0.21218	27,15	0.45787	42,15	0.67237	57,15	0.84104	72,15	0.95240	87,15	0.99885
47	0.01367	12,30	0.21644	27,30	0.46175	42,30	0.67559	57,30	0.84339	72,30	0.95372	87,30	0.99905
48	0.01396	12,45	0.22070	27,45	0.46561	42,45	0.67880	57,45	0.84573	72,45	0.95502	87,45	0.99923
49	0.01425	13	0.22495	28	0.46947	43	0.68200	58	0.84805	73	0.95630	88	0.99939
50	0.01454	13,15	0.22920	28,15	0.47332	43,15	0.68518	58,15	0.85035	73,15	0.95757	88,15	0.99953
51	0.01483	13,30	0.23345	28,30	0.47716	43,30	0.68835	58,30	0.85264	73,30	0.95882	88,30	0.99966
52	0.01513	13,45	0.23769	28,45	0.48099	43,45	0.69151	58,45	0.85491	73,45	0.96005	88,45	0.99976
53	0.01542	14	0.24192	29	0.48481	44	0.69466	59	0.85717	74	0.96126	89	0.99985
54	0.01571	14,15	0.24615	29,15	0.48862	44,15	0.69779	59,15	0.85941	74,15	0.96246	89,15	0.99991
55	0.01600	14,30	0.25038	29,30	0.49242	44,30	0.70091	59,30	0.86163	74,30	0.96363	89,30	0.99996
56	0.01629	14,45	0.25460	29,45	0.49622	44,45	0.70401	59,45	0.86384	74,45	0.96479	89,45	0.99999
57	0.01658	15	0.25882	30	0.50000	45	0.70711	60	0.86603	75	0.96593	90	1.00000
58	0.01687	15,15	0.26303	30,15	0.50377	45,15	0.71019	60,15	0.86820	75,15	0.96705		....
59	0.01716	15,30	0.26724	30,30	0.50754	45,30	0.71325	60,30	0.87036	75,30	0.96815		....
60	0.01745	15,45	0.27144	30,45	0.51129	45,45	0.71630	60,45	0.87250	75,45	0.96923		....